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CONTRACTING ORGANIZATION:
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The description of work accomplished is presented under four headings: i) collection of samples; ii) establishment of a database; iii) preparation and distribution of samples; and iv) development of assays relevant to breast cancer.

i) We have established protocols for collection and utilization of tissues at Yale and at seven off-site hospitals. We also have initiated or obtained IRB approval in all these sites, and have trained the technical personnel.

ii) We have established protocols to implement the database proposed in the original application, and initiated a data exchange program with the Yale Cancer Center Breast Program and the Yale-New Haven Hospital Tumor Registry.

iii) We have collected a total of 204 samples of breast carcinoma, 105 tumor samples having matched normal tissue. Since the beginning of operations, we have provided 819 tissue derived products to 32 investigators.

iv) We have developed assays to assess the status of p53, Ki-ras, cyclin D-1 (PRAD), NEU, microsatellite variability, and p16 (MTS).

In addition, we participate in a working group addressing the ethical and legal issues raised by DNA repositories storing samples linked to medical information concerning individual patients.

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Table of Contents

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Introduction

The Program for Critical Technologies in Breast Oncology (PCTBO) establishes a core technical and tissue procurement resource that: i) maximizes access to human breast tissues and tumor DNA for basic investigators; ii) facilitates the application of molecular technologies in clinical breast oncology; and iii) makes such technologies routinely available to clinical investigators. This program builds on Yale's existing Program for Critical Technologies in Molecular Medicine and the Tissue Procurement Core Facility of the Yale Cancer Center, and complements in a rigorous and planned way the Yale Tumor Registry and Yale's Rapid Case Ascertainment System.

The PCTBO enhances the investigational availability and utilization of human breast tissues by centralizing the collection, banking and distribution of fresh and frozen tissue samples, and by providing DNA, RNA, tissue sections and ready-to-use nucleic acid blots. Samples of frozen breast tissues are being collected in an ongoing basis from major hospitals in Connecticut, representing a population base of over three million people. Clinical data with pathologic evaluation is maintained for each sample.

The application of molecular technologies to clinical breast oncology is enhanced by development and implementation of methods for the routine analysis of oncogenes and tumor suppressor genes of relevance to breast cancer. Initial PCTBO efforts in this area focus on immunohistochemical, biochemical, and molecular biologic assays defining the functional status of p53, Ki-ras, and neu, as well as genes of emerging significance.

Body of Report

We have organized the body of our annual report to follow the tasks delineated in the original proposal's Statement of Work: i) collection of samples; ii) establishment of a database; iii) preparation and distribution of samples; iv) development of asssays relevant to breast cancer.

Task 1) Centralize collection of fresh, fixed, and paraffin embedded breast tissue samples from patients treated at Yale New-Haven Hospital and other hospitals in Connecticut.

1a) Establish standardized protocols for procuring fresh frozen and paraffin-embedded tissue samples of breast cancer cases from off-site institutions

We have defined and implemented protocols to obtain fresh frozen tissue. (See Appendix 1). Given the present trend in diagnosis and therapy of breast masses we have taken two approaches. The first is to continue with an aggressive prospective acquisition of samples of tissue that are prepared and embedded in OCT medium such that frozen sections can be cut from the samples. If extra tissue is still remaining, additional aliquots are bulk-frozen in tissue cassettes in liquid nitrogen. We have found that many investigators' requests can be satisfied with "thick frozen sections" preceded and followed by thin sections to verify the tissue status and structure. We estimate that a tissue sample prepared for sectioning yields a minimum of 200 thin sections (6 μm) and on the average provides five samples from which DNA can be extracted and analyzed. The larger tumors, from which "bulk" specimens are available, can be used for nucleic acid or protein extraction after grinding the frozen tissue under liquid nitrogen in a mortar and pestle to obtain a fine frozen powder.

The second approach we have taken is applicable to samples such as small biopsies for which excess tissue is often not available from a gross specimen. In these cases, we can obtain extra

frozen sections at the time a clinical section is done during an intra-operative consultation. Once the pathologist has concluded the consultation on a frozen specimen, an extra 10 sections are cut and stored on slides that are kept frozen at -80° C for future use. The remainder of the frozen specimen is handled as usual by the pathologist. We should note specifically that these slides are handled in the same manner as are the larger samples: no research use is made of any material before at least one week has elapsed after the final pathology report has been issued.

1b) Provide off-site training on fresh tissue handling to personnel in participating institutions
1c) Expand routine tissue catchment to include at least five of Connecticut's largest hospitals, including most or all of those listed as phase I

During the second half of the 1994-95 period we have visited seven institutions in the state of Connecticut and initiated the off-site collection program. The pathologists at these sites have been informed of the program and the technical personnel have been instructed in collection protocols. In all sites visited a liaison pathologist and technician have been designated and we have begun the process of obtaining Institutional Review Board approvals. As of June 30, 1995 the following hospitals have begun collection and/or are in the process of obtaining IRB approval to participate:

Table 1: List of Off-site Hospitals

Hospital & City	Date of Initial Contact	Contact Pathologist/ Department Chair/ Collection Technologist	Date of IRB approval
Yale-New Haven Hospital, New Haven, CT	8/94	Darryl Carter, M.D. Jon Morrow, M.D., Ph.D. Leticia deDios, M.D.	7/94
Veterans Hospital, West Haven, CT	9/94	Robert Homer, M.D. Gary Stack, M.D. Leo Kelley, P.A.	in process
Greenwich Hospital, Greenwich, CT	10/94	Richard Eisen, M.D. Stephen Gray, M.D. Claire Arkemone, H.T.	7/95
Bridgeport Hospital, Bridgeport, CT	11/94	Gustave Davis, M.D. Gustave Davis, M.D. Pam Thomas, P.A.	in process
Danbury Hospital, Danbury, CT	3/95	Raoul Braza, M.D. Ramon Kranwinkel, M.D. Mary Davis, Ph.D.	in process
Norwalk Hospital, Norwalk, CT	4/95	Gustavo Reynoso, M.D. Gustavo Reynoso, M.D. Margaret Keane, H.T.	in process
Hospital of St. Raphael, New Haven, CT	5/95	Paul Fiedler, M.D. Romeo Vidone, M.D. Gail Barricelli, M.T.	11/94
The Stamford Hospital, Stamford, CT	scheduled 8/95	Michael Lotz, M.D.	
The Waterbury Hospital, Waterbury, CT	scheduled 8/95	William G. Frederick, Ph.D.,M.D. Moses K. Lieberman, M.D.	
St. Mary's Hospital, Waterbury, CT	planned 9/95	Dwight Miller, M.D.	
The Griffin Hospital, Derby, CT	planned 10/95	Stephanie Wain, M.D.	

1d) Develop a mutual tissue sharing arrangement with Hartford Hospital (a University of Connecticut affiliate) to enhance breast cancer research at both the University of Connecticut and Yale University.

[Note: "Managed care" has somewhat modified the relationship of Yale-New Haven Hospital with Hartford Hospital and introduced an element of caution that has resulted in a slow-down of the planned tissue sharing program. However, we are confident that the dialogue between our institutions will be productive as state-wide roles and alliances are more clearly defined for both organizations.]

Task 2) Establish comprehensive database linked to the CTBO tissue bank & designed to support correlative multidisciplinary studies that utilize tissue samples

2a) Establish on-line a database incorporating the data items outlined
2b) Establish computer network interfaces, protocols, and procedures to assure collection of the information outlined

We have established the protocols to implement the database as proposed in the original application. The data files are resident in a Macintosh PowerPC 7100 fileserver and have been created using FileMaker Pro software. The Critical Technologies fileserver has been interfaced with the Novell network existing in the department of Pathology. Besides giving the PCTBO access to routine information in the clinical files, the connection to the departmental network provides access to a digitized image datafile of gross and microscopic images pertaining to specimens accrued by the PCTBO. In addition, two state-of-the-art Roche imaging work stations equipped with advanced digital cameras allows the PCTBO to capture high quality images of the specimens stored or provided to investigators.

2c) Implement the collection of such information on samples collected at YNHH

The recent reorganization of the Clinical Research Office at the Yale Cancer Center [YCC] and the establishment of a Breast Cancer Research Program within the YCC will greatly facilitate the acquisition of information pertaining to the patients. A research nurse has been assigned to the YCC Breast Program and will assume the task of obtaining and managing the patient-related information. These data will be transfered to the PCTBO database at regular intervals, and anonymous information describing samples can then be distributed at the time the samples themselves are given to investigators.

In addition, we have evaluated the data collection system of the Yale Tumor Registry. We have concluded that the most efficient mode of transfer will require extensive re-programming of both the Tumor Registry's and PCTBO's software to create a on-line link. We have applied for supplemental funding to partially support these efforts.

Task 3) Prepare and distribute breast tissue samples from the CTBO repository to investigators

3a) Establish standard protocols for tissue requests and distribution

Protocols for tissue request and distribution have been established and implemented. (See appendices 2 and 3 for a registration and sample request forms.) We have worked closely with the Yale Human Investigation Committee to insure that all programs involving research use of samples of archival or fresh frozen human breast tissue are channeled through the PCTBO. To date, most

of the demand has been for archival paraffin tissues; as frozen samples are accumulated, these sample sets are increasingly being requested.

3b) Establish multidisciplinary review board to establish principals for prioritizing requests for rare breast lesions

A process for review, monitoring, and development of clinical research studies in cancer was established in September 1993 by the Yale Cancer Center. This process provides internal peer review to commit resources to clinical studies. One component of this process, the "Clinical Studies Group," can address any conflicts that may arise in the course of distribution of breast tissue samples and products derived therefrom. The composition of the Group (see appendix 4) guarantees a balanced arbitration process.

3c) Distribute fresh, frozen, fixed, and paraffin embedded breast tissue to investigators

Over the past year of collection, we have obtained 589 parts from breast cases. A total of 2289 individual samples were collected. Of these, frozen tissue accounts for 103 cases, with a total of 1529 samples. The others were collected and distributed in other forms, including fresh or fixed. A detailed list of cases collected is included in appendix 5.

Numbers of breast cases that have been collected have dramatically increased in the past year, due to the support of the PCTBO and accompanying efforts to target breast tissues specifically. Table 2 shows data for numbers of breast cases frozen; an almost 300% increase in numbers frozen is evident since the inception of the PCTBO. This table does not include samples that were distributed as either fresh or fixed tissues, but reflects the numbers of cases available for further and future utilization by investigators.

Table 2. Breast tissue, frozen tissues collected by year
Total numbers of cases, and numbers of cases with matched tumor and normal tissues
(Each case has multiple samples associated with it)

	1993		1994		(1995 total year projections)	
	total	matched	total	matched	total	matched
Breast	52	30	73	39	204	105

Overall, since the inception of the PCTBO, we have provided 15 investigators with 923 samples of fresh, fixed, and frozen breast tissues. (Appendix 6). However, in the past month (July 1995) alone, we have received an additional number of inquiries about our frozen bank from some 8 more investigators who were informed of this resource through the Yale Cancer Center's Breast Cancer Research Program. As word of the PCTBO continues to spread, we anticipate strong growth in our distribution list.

In the case of paraffin-embedded breast tissues, liaisons with the Rapid Case Ascertainment Shared Resource of the Yale Cancer Center, and with the Yale-New Haven Hospital Tumor Registry, as well as searches of the Pathology Department's Information System have allowed identification of breast cancer cases by approximately a dozen research groups. The process of identifying specific paraffin blocks containing tumor has begun, with sections cut and examined for over 2000 blocks. The status of the tumor and its characteristics have been ascertained by the pathology fellow associated with the Critical Technologies program.

3d) Establish methods for distributing DNA, RNA, and blots suitable for hybridization studies

Extraction of DNA from frozen sections and from archival paraffin tissues is routinely done in our laboratories. (See appendix 7). Extraction of standard quality RNA from thick (10-20 μm) frozen sections (and in some cases from paraffin blocks) is also routinely accomplished. Extraction of very high quality RNA is in the process of being developed and tested. Our preliminary results suggest that obtaining very high quality RNA that will allow sophisticated quantitative display of the genes expressed in a tissue sample requires immediate and instant deep freeze of the tissue in the operating room. Given the minimal size of the breast biopsies and the lesions contained in the tissue, collection of very high quality RNA will be limited to large tumor specimens.

3e) Inventory and review samples in paraffin archive dating back to 1915

Pathology final reports dating 1915 to 1983 have been entered on computer-readable media and are ready to be loaded onto the Pathology Information System. The current VAX 11785 mainframe computer will be replaced with a DEC AlphaServer 2000 to yield a much faster system. The hardware for the conversion is in, and software will be installed, de-bugged, and functioning by end of summer 1995.

The accomplishment of this task will make accessible longitudinal data for a total period of 80 years, 1915 to 1995. The data will be free-text searchable on several fields, for instance, tissue source and final diagnosis fields. Because most searches are performed from the existing pathology computerized records dating back only to 1983, this new inventory will make an additional 460,000 pathology cases available for research.

Refinements of the system will definitely occur such that older cases will be merged with current patients. This merging will ensure that older specimens will be able to be identified for patients who are in the current database, 1983 to present. Overall, when the inventory and computerization work is done, complete records on over 900,000 cases will be able to be searched; all of these cases have paraffin blocks available in the archives. Of these cases, our available data from the Yale Tumor Registry indicate that 10,750 are of breast cancer, including both invasive and in situ.

The paraffin archives themselves have likewise been improved. Ongoing work will be finished by the end of August 1995 on complete reorganization of over 3 million paraffin blocks. This work will eventually have consumed over 3000 meticulous person-hours of time, and involves the physical sorting and refiling of all blocks. The warehoused locations of the paraffin blocks have been consolidated from 3 separate sites to 2 closer, climate-controlled sites. These overall improvements have made a huge difference in ease of use of this most valuable resource.

Task 4) Develop and offer on a minimal fee-for-service basis routine molecular and histologic tissue analyses of relevance to breast cancer. Of particular interest are assays that can be carried out on minimal tissue samples.

4a) Implement FASAY assay for p53 function in breast cancers

Approximately 80% of p53 mutations in tumors are missense mutations (1), scattered throughout the coding region, with the majority occuring between codons 100 and 300 (2). The critical function of p53, which is closely linked with its tumor suppressor activity, is the ability to activate transcription of specific genes. The promoter regions of these genes contain p53 binding sites. All mutant forms of p53 lack the transcriptional activity of the wild-type protein.

A functional assay of the transcriptional activity of p53 from a specific tumor has been established (3-6) by taking advantage of the efficient mechanisms of gap repair by homologous recombination in the yeast, *S. cerevisiae.* This assay has identified all tested cancer-associated mutant forms (Δ59 with frameshift, 143A, 156P, 173 M, 175H, 214R, 238S, 245C, 248W, 248Q, 249S, 252P, 258K, 272L, 273H, 281E, 282W, 307stop) as defective in transcriptional activity (4-6).

Methods

Using reverse transcription and the polymerase chain reaction, full length p53 cDNA is amplified from poly-A RNA isolated from a biopsy or resection specimen. To minimize the introduction of base mismatches during the amplification process, the high fidelity, proof-reading Pfu polymerase is used.

A yeast expression vector (pSS16) containing a selectable marker (LEU2, conferring leucine prototrophy) is linearized with cut ends homologous with the p53 coding sequence. The yeast are co-transformed with the p53 PCR product and the linearized vector. Under selection in the absence of leucine, clones are identified which contain a plasmid which has been repaired by homologous recombination with a p53 PCR product. Thus, the p53 alleles in a patient's tumor can be constitutively expressed in S. cerevisiae. A yeast strain (yIG397) is used which has an integrated sequence containing a reporter gene (ADE2) downstream from a minimal promoter containing three copies of the RGC p53 binding site. Thus, screening on medium containing a limiting adenine concentration identifies colonies that contain transcriptionally active p53 (ADE2+ yeast grow as normal white colonies) and colonies that express non-functional p53 protein (ade2- yeast form red colonies in limiting adenine media due to the accumulation of an intermediary compound in adenine synthesis). As yIG397 is a centromeric yeast plasmid, individual colonies contain a single copy of the plasmid and thus express a single p53 allele. The fraction of total colonies that are white reflects the fraction of the amplified alleles which have transcriptional activity.

Preliminary Results

Conditions for amplification of the full length p53 coding sequence and for transformation of yIG397 have been optimized. When high quality RNA is available, the p53 coding sequence is reliably amplified. Positive and negative controls give the predicted results as follows (see Figures 1 and 2): white colonies are obtained when a plasmid containing a wild type p53 sequence is used as a template for amplification; in contrast, exclusively red colonies are seen on assay of p53 cDNA from A431, a squamous cell carcinoma cell line which has previously been characterized as expressing only mutant p53 is assayed.

A representative test sample: the p53 coding sequence was amplified from an clinical specimen resected at Yale. Transformation of the yeast with linearized pSS16 and the amplified p53 cDNA yielded exclusively red colonies, indicating that the carcinoma expressed only mutant p53

4b) Establish protocols for the quantitative analysis of ras, prad, and neu

4b1) Analysis of RAS oncogene

Primers encompassing regions of the Ki-ras and Ha- ras genes that are often mutated have been designed and successfully tested. The amplification product is analyzed by SSCP and we have established a diagnostic pattern for each of the mutations in codons 12 and 13 of Ki-ras. (See Figure 3). We will now proceed to test the same approach for Ha-ras.

4b2) Analysis of PRAD-1 (Cyclin D1)

PCR

PRAD-1 primers were designed to flank an intron-exon boundary and and allow selective PCR of genomic DNA sequence (unpublished 5'-GCGGGACGTGGACATCTGAG-3' and 5'-AGAGATGGAAGGGGGAAAGA-3'). Five points, in the linear range between 20-30cycles of PCR, are evaluated for matched "tumor" and "normal" samples and parallel amplification of HLA (5'-GTGGTGTAAACTTGTACCA-3' and 5'-GGTAGCAGCGGTAGAGTT-3') is used as a control. Input target is 20 ng genomic DNA.

Gel Analysis

Acrylamide gel analysis (12%; 29:1 acrylamide:bisacrylamide in a continuous 1% TBE Buffer system) is based on co-electrophoresis of PRAD-1 and HLA amplification products in the same well. After staining with Ethidium bromide or SYBR-Green I, densitometry allows a comparison between the PRAD-1/HLA ratio of product in tumor with that present in the normal sample. *In vivo* PRAD-1 gene copy amplification >3x normal can be detected.

4b3) Assay for NEU oncogene function

The neu/erbB-2/HER-2 receptor Tyrosine kinase is frequently amplified and/or overexpressed in mammary carcinoma, and shows great potential as a prognostic factor and therapeutic target. David Stern has developed a unique serological reagent, an antibody that detects activated (Tyrosine-phosphorylated) neu and not other Tyr phosphoproteins (7). This enables direct staining of tumor tissue and quantification of the number of activated receptors. This novel antibody, capable of specifically detecting only *activated* receptors, should provide a much better test of neu function than simply quantifying receptor number, as is done with conventional anti-peptide antibodies. The new test should improve the weak prognostic associations of neu overexpression, and should help identify the subset of patients that would benefit most from neu-targeted therapies. The latter is of crucial importance since Phase III passive immunotherapy trials targeting neu will begin shortly.

Ongoing work performed by Michael DiGiovanna includes a survey of more than 200 breast carcinoma *in situ* specimens for association of phosphorylated neu with prognostic indicators, and a survey of a consecutive series of 250 infiltrating ductal carcinomas. The results for the former survey are already encouraging in showing a strong association of phosphorylated neu with other prognostic factors.

4c) Establish protocols for mini and microsatellite repeat variability

We have adapted established methods (8,9) for use in the Critical Technologies Program by eliminating the need for use of radioactive probes. Our overall aim is establishment of clinically useful tools, and non-radioactive protocols have a tremendous advantage in these circumstances. PCR primers for 5 trimeric and tetrameric short tandem repeats were utilized in standard protocols. (see Table 2) The fact that these 5 loci are highly polymorphic, and able to be reliably PCR amplified from samples as small as thin paraffin sections on slides, make them ideal for analysis of STR variability.

Table 2: Polymorphic short tandem repeat loci used for studies (8,9)

locus and STR	chromo-some	PCR primers (5'-3')	product length (BP)	gene
HUMFABP[AAT]$_n$	4q31	gtagtatcagtttcatagggtcacc cagttcgtttccattgtctgtccg	199-220	intestinal fatty acid binding protein
HUMARA[AGC]$_n$	Xcen-q13	tccagaatctgttccagagcgtgc gctgtgaaggttgctgttcctcat	261-312	androgen receptor
HUMTH01[AATG]$_n$	11p15.5	gtgggctgaaaagctcccgattat attcaaagggtatctgggctctgg	183-207	tyrosine hyroxylase
HUMRENA4[ACAG]$_n$	1q32	agagtaccttccctcctctactca ctctatggagctggtagaacctga	251-271	renin
HUMHPRTB[AGAT]$_n$	Xq26	atgccacagataatacacatcccc ctctccagaatagttagatgtagg	263-299	hypoxanthine phosphoribosyltran sferase
GABARB1	4p	tgatagctagaaagctagcaag gctcattaaacactgtgttcct	139-163	γ–aminobutyric acid receptor

4d) Establish routine research histochemistry and immunohistology service for breast tissue

We have been working extensively on histologic stains specifically useful for breast tissues, and a routine service facility is well-estaablished. For protocol see appendix 9; order form is shown in appendix 3) However, during the course of routine work utilizing immunostaining for estrogen receptor, we encountered extreme difficulty in staining on older cut slides. After much trial and error, we discovered that paraffin-embedded tissues stored as 3 to 5 μm sections on glass slides lose reactivity to many antibodies, including estrogen receptor. We have evidence that other antibodies likewise affected include neu, progesterone receptor, and p53. A recent article confirms our observations. (10) Storage of unstained slides at room temperature for as little as one week causes decrease in staining intensity, with total loss of reactivity sometimes occuring after 4 weeks. This loss of reactivity occurs only after sectioning: storage of the intact paraffin block does not seem to have any ill effect on antigenicity.

To address this problem, we tried various staining protocols on both stored and freshly cut sections. Two protocols were very effective: the use of recent antigen-retrieval method of pressure-cooking (11), and use of a commercially available "one-step" method of staining (Dako EnVision system, antibody and HRP coupled to an inert polymer backbone; or "universal" secondary with poly-HRP). Both of these techniques can be applied to any immunohistochemical staining protocol. Pressure-cooking can also be utilized in immunoblots.

Other progress not stated in original statement of work:

1. p16 Analysis

20ng genomic DNA is used as target for highly specific `touchdown' PCR using the following primers: 5'-GAAGAAAGAGGAGGGGCTG-3' and 5'-GCGCTACCTGATTCCAATTC-3'. (12). Products are checked for mutation and loss of heterozygosity by direct sequence analysis or

SSCP analysis (+/- restriction digest of amplicon). (Specific methods are described in a manuscript in preparation.)

2. SSCP screening for p53 mutation

Paraffin-embedded archival tissues are not suitable for the functional assay of p53 (see preceding section) since the procedure requires good quality RNA. For such specimens, however, the mutational status of p53 can be accessed by PCR-amplification of selected exons (exons 5 to 9) and gel analysis of the products under denaturing conditions. See appendix 10.

3. Work towards establishing ethical guidelines and policy at Yale for use of human tissue samples in both anonymous and patient-linked studies.

The currently approved IRB protocol under which PCTBO operates is limited to anonymous collection and use of tissue samples. This restriction implies that no followup data (e.g., patient survival and other outcome data) or laboratory results from study of tissues can be accrued in the database to further describe each individual specimen. To address this problem, Yale's IRB, the Human Investigation Committee, has assembled a working group, the "Tissue Subcommittee," specifically charged with developing functional guidelines for resolving the difficult ethical questions raised by the collection, storage, and use of human tissue specimens linked to patient identity.

Conclusions

With one exception (Task 1d), we have accomplished all goals presented in the original application for the first year of the project. The infrastructure to accomplish these goals has been established, has begun to function, and provided proof of concept that the wide range of capabilities encompassed by the PCTBO can facilitate research and cooperation at a large academic center such as Yale. As a specific example, Dr. Richard Hochberg has been a recent active collaborator reaping the benefits of the PCTBO. It is only the new ability to procure appropriate breast tissue and assemble the supporting medical and technical expertise that has allowed his sophisticated research to be rapidly and successfully executed. His project involves the analysis of breast tumor specimens for the activated estrogen receptor.

The analysis of breast tumor specimens for the estrogen receptor is routinely performed in many laboratories in order to determine whether the cancer may be responsive to antiestrogen therapy. Since a large portion of tumors that contain the estrogen receptor do not respond to such therapy, other markers of estrogen action, usually the progesterone receptor, are also measured. Recently Dr. Hochberg and his lab have developed a method that allows not only the quantitative determination of the estrogen receptor, but more importantly, the specific measurement of the activated estrogen receptor (ERAct) (13).

Since it is the determinant of transcriptional activation, the ERAct can provide an important new indicator of estrogenic control in breast cancer. Quantification of the activated receptor can illuminate several important parameters of estrogen action: whether the level of estrogen provided through the circulation is sufficient to activate the receptor; or even if it is inappropriately high, possibly an indication that local production in the breast may be supplying estradiol directly to the tumor as has been postulated; or an inability of the receptor to bind to estrogen response elements (ERE) of genes because of receptor mutation or other factors. Thus the presence of the ERAct confirms several important features of estrogenic stimulation; 1) the presence of concentrations of estrogens (regardless of their nature) sufficient for estrogenic stimulation. 2) the ability of the receptor to bind both estrogen and the genomic estrogen response element. Thus the ERAct in a

tumor could be a very good predictor of an antiestrogenic response for it demonstrates whether the tumor is under estrogenic stimulation. The information provided goes well beyond the immunological or ligand binding detection of the receptor. The procedure that Dr. Hochberg has developed for the measurement of the activated receptor involves autoradiography and densitometric analysis of the receptor labeled in vitro (in situ) through the binding of an [^{125}I]estrogen receptor ligand, 11-beta-methoxy-16-alpha-[^{125}I]iodoestradiol. He also developed an unusual ligand (14) which has a high affinity for the estrogen receptor, and which because of its high specific activity, 2,200 Ci/mmol, allows autoradiographic analysis within 24 hours. This procedure is very sensitive; and cleanly differentiates between the ligand activated receptor and the unoccupied receptor. He intends to compare the clinical histochemical analysis of estrogen receptor and progesterone receptor with autoradiography of the total estrogen receptor and ERAct in human breast cancer specimens.

A second specific example of the power of the PCTBO is reflected in the support given to Dr. Tong Zheng's epidemiological study of PCB's in breast cancer. He and Dr. Robert Dubrow have requested collection of breast fat from study subjects who undergo breast surgery. Fat is not normally collected, and we have been able to organize and orchestrate the collection of this tissue upon which Dr. Zheng's whole study relies. We have coordinated extensively with the pathology resident and attending physicians involved in all stages of the diagnostic process, from gross description to final report, and collect both fresh and formalin-fixed fat from cases identified through a computer report that was generated with input from PCTBO personnel.

The next years will confirm and consolidate advances made in this first year of the PCTBO. The PCTBO has been enthusiastically received by members of the Yale Cancer Center's Breast Cancer Research Program, and investigator utilization of the PCTBO is likely to increase over the next two years.

Other important future work includes the definition of policy and ethical guidelines for research use of human tissues such that scientific progress can continue without compromise of patient rights. We have applied for supplemental funding to help support our efforts in this area.

Manuscripts in preparation will detail the processes, protocols, and experience we have acquired in the initial operations and management of a complex and multipartite resource for support of biomedical research. It is hoped that our dissemination of this information will stimulate cooperative interactions between the PCTBO and similar programs at other institutions.

References

1. Friend, S *Science* **265**, 334-5 (1994).

2. Zambetti, G.P. & Levine, A.J. *FASEB J* **7**, 855-65 (1993).

3. Scharer, E. & Iggo, R. *Nucleic Acids Res* **20**, 1539-45 (1992).

4. Ishioka, C., *et al Nat Genet* **5** 124-9 (1993)

5. Frebourg, T., *et al. Cancer Res* **52**, 6976-8 (1992).

6. Flaman, J-M., *et al Proc Natl Acad Sci USA* **92**, 3963-7 (1995)

7. DiGiovanna, M.P., and Stern, D.F. 1995. Activation state-specific monoclonal antibody detects tyrosine phosphorylated p185 neu/erbB-2 in a subset of human breast tumors overexpressing this receptor. *Cancer Res.* 55:1946-1955)

8. Edward, A., Hamond, H.A., Jin, L., Caskey, C.T., and Chakraborty, R. 1992. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics* 12: 241-253.

9. King, B., Lichtenstein, A., Berenson, J., and Kacinski, B. 1994. A polymerase chain reaction-bases microsatellite typing assay used for tumor cell line identification. Am. J. Pathology 144: 486-491.

10. Bromley, C.M., Palechek, P.A., and Benda, J.A. 1994. Preservation of estrogen receptor in paraffin sections. *J. Histotechnology* 17: 115-118.

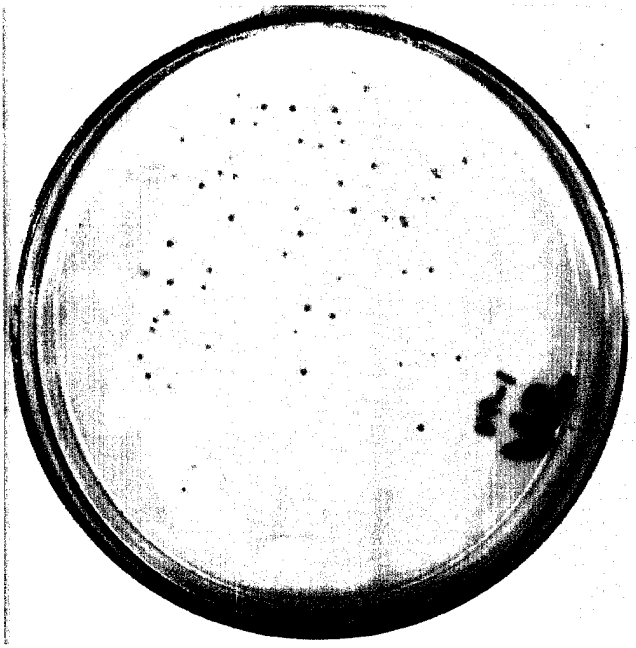
11. Norton, A.J., Jordan, S., and Yeomans, P. 1994. Brief high-temperature heat denaturation (pressure-cooking): A simple and effective method of antigen retrieval for routinely processed tissues. *J. Pathology* 173: 371-379.

12. Caldas C., Hahn SA, daCosta LT, Redston MS, Schutte m, Seymour AB, Weinstein CL, Hruban RH, Yeo CJ, and S Kern (1994) Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nature Genetics* 8:27-32.]

13. Yuan H, Bowlby DA, Brown TJ, Hochberg RB, MacLusky NJ. 1995 Distribution of occupied and unoccupied estrogen receptors in the rat brain:Effects of physiological levels of gonadal steroids. *Endocrinology* 136:96-105.

14. Zielinski JE, Yabuki H, Pahuja SL, Larner JM, Hochberg RB. 1986 16-alpha-[^{125}I]Iodo-11-beta-methoxy-17-beta-estradiol: A radiochemical probe for estrogen sensitive tissues. *Endocrinology* 119:130-9.

(1) The yeast, yIG397, were co-transformed with linearized pSS16 and the amplified p53 cDNA. After incubation for 2 days at 35C in media containing limiting adenine, exclusively red colonies were identified.



(2) By contrast, when yIG397 was transformed with pSS16 and wild type p53 cDNA, exclusively white colonies were identified.

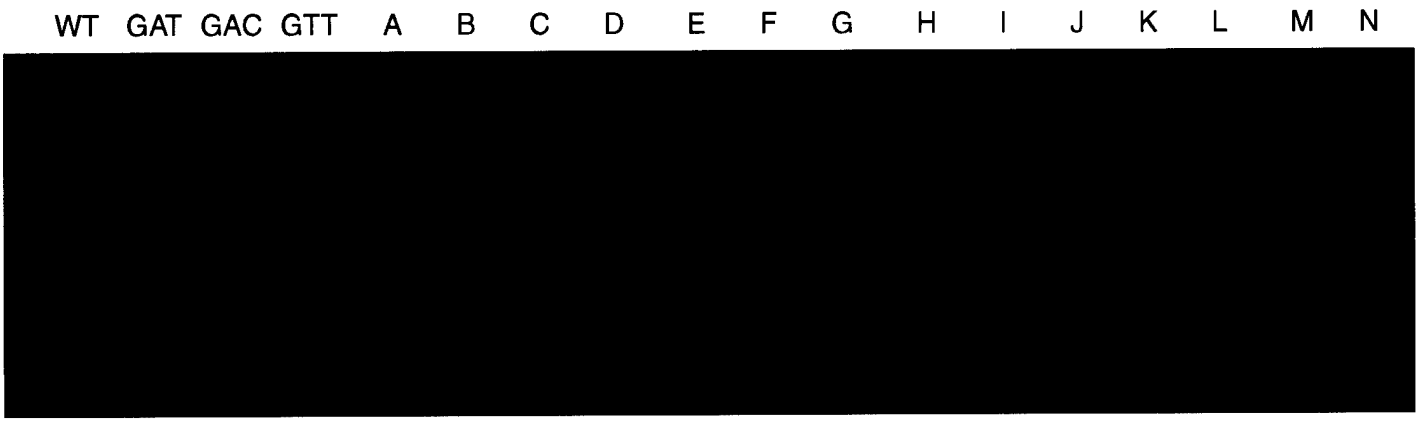


Fig. 3

SSCP patterns of Ki-ras mutations.
(Lanes B to M represent analyzed tissue samples.)

Appendices

Appendix 1: Protocols for Freezing Tissue Specimens

Program for Critical Technologies in Molecular Medicine
Yale University Department of Pathology
203-737-4198 or 203-785-5879

Brief description of the research. The Program for Critical Technologies received a multi-year grant from the U.S. Army Research and Development Command to collect human breast tissue samples and make the specimens available to basic and clinical researchers. We are hoping to collect specimens from many major hospitals in Connecticut, and can in some instances supply these off-site locations with necessary equipment for freezing tissues.

What to collect? Collect "everything:"

all tumor specimens
other pathologic specimens
matched normal (cellular) tissue from each case, such as skin, muscle, etc.

How to collect

1) Collect or save any tissue needed for diagnosis

2) Collect both tumor/pathologic and normal tissue from the excess specimen and freeze in separate OCT molds. If the specimen is large, collect multiple samples in individual molds.
OCT molds [large molds, Miles Tissue-Tek #4557, 25x20x5 mm]
Label each mold with
a) the part number
b) the case number
c) N "normal" or T "tumor or other pathology" for example:
d) a sequential number starting with 1 for each case
2)S93-123 N1; 2)S93-123 T2; 2)S93-123 T3, etc.

Put tissue **(maximum 1 cm x 1 cm x 0.5 cm thick)** in mold with OCT
Freeze in isopentane bath by holding mold at surface of the liquid
Label (as above) Bitran bags (3" x 6" in size, #4741-S) along the top of one *long* side.
Remove molds from isopentane, allow to drain briefly, and put molds in Bitran bag(s). If there are more than 2 samples from one case, separate the "T" from the "N" samples in separate bags.
Put samples into -80°C freezer.

Tissue collection triage Timeliness of collection. Although fresher tissue is definitely better, other tissue can be useable, especially for DNA work, and should be collected. Collect tissue with the following priorities for freshness:

a. Tissue arriving for a frozen section. [Note: extra frozen section unstained slides are useful; store in slide container in a Bitran bag at -80°C]
b. Tissue hand-carried from the OR.
c. Routine specimens arriving at surgical pathology.
d. Autopsy specimens.

Program for Critical Technologies in Molecular Medicine

General Information Form, Yale Investigators

Please return this form to Brady Memorial Lab B250. Call Dr. Christine Howe for information, 737-4198.

CONTACT PERSON:

First name | *Last name* | *Degree(s)* | *Position at Yale* | *Application date*

Department | *Building and room* | *Phone 1* | *Phone 2* | *Beeper* | *FAX*

Brief description of study:

COLLABORATORS IN THIS STUDY:

First name | *Last Name* | *Department* | *Building and room* | *Phone*

PRINCIPAL INVESTIGATOR:

O Yes
O No

First name | *Last name* | *Degree(s)* | *Position at Yale* | *YCCC member?* | *Email Address(es)*

Department | *Building and room* | *Office phone* | *Lab phone* | *Beeper* | *FAX*

GRANT AND ACCOUNTING INFORMATION: O "Peer-reviewed" O "Non-peer-reviewed" O Not grant funded

If this study is funded by a grant, please complete the next line. If you will be paying for services, also complete the charging information to the right:

Yale charging number

Granting agency | *Grant number [not charging #]* | *Title of grant*

ALL REQUESTS FOR HUMAN MATERIAL AND/OR PATIENT INFORMATION: O anonymous O linked

I certify that I have Human Investigation Committee approval for all human samples and any descriptive information I have requested. (Yale HIC must approve all human research even if it is exempt from coverage by NIH regulations.)

HIC approval /renewal date ..... *Expedited review?* ☐ Yes *and/or HIC number* .....

Signature of Principal Investigator ..... *Date* .....

ALL REQUESTS FOR HUMAN BIOLOGICAL MATERIAL:

I certify that I am aware of the biohazards associated with use of human biological material, and I assume responsibility to ensure compliance with all Federal, State, Yale-New Haven Hospital, and Yale University safety regulations regarding biohazardous human material, including biohazard labelling, pathogen training requirements, and notification about availability of Hepatitis B vaccination. As the principal investigator, I acknowledge that I am responsible for ensuring that all staff act in compliance with these safety regulations and have been properly trained. I agree that I am assuming responsibility for the use, handling, storage, disposal, or distribution of the biological material, and that the Program for Critical Technologies, Yale Department of Pathology, and Yale-New Haven Hospital have no further responsibility for the use of the biological material.

Signature of Principal Investigator ..... *Date* .....

Info 5/3/95

Appendix 3: Request form

Research Histology & Tissue Products

Program for Critical Technologies in Molecular Medicine
Yale University Department of Pathology, Lauder Hall 202
785-5879 (lab); 737-4198 (office); 737-1064 (fax)

ORDERED BY **Date**.....

Contact Person..... *(First name)* *(Last name)*

Building and room..... **Phone**.....

PI..... **Department**.....
(First name) *(Last name)*

Yale Charging Instructions.....

-or-

Billing Address.....

.....

.....

ITEMS ORDERED **Date Needed**.....

 # Samples..... **Color of cassettes (optional)**.....

Starting Material *(Check only one)*
☐ Fresh/Fixed Tissue ☐ Pathology Paraffin
☐ Investigator's Paraffin ☐ PCTMM Paraffin
☐ Investigator's Frozen ☐ PCTMM Frozen
☐ Section/Slide

Quant. per Sample	Service Code	Service Description	Comments
.....	.....	.....	
.....	.....	.....	
.....	.....	.....	
.....	.....	.....	
.....	.....	.....	

Please list samples you submit/request (use separate sheets if necessary)

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

Departmental Authorization (optional)..... **Date**.....

Critical Technologies use only

Hours for job ..... **CT Order #**.....

Date Received..... **Received by**..... *(initials)* ☐ Histology ☐ Immuno ☐ Special

Date Complete..... **Completed by**..... *(initials)* ☐ Histology ☐ Immuno ☐ Special

RMS2 RH 7/5/95

№ 2772 Pink copy—Requester Yellow copy—Critical Technologies White copy—Accounting

Appendix 4: Composition of the "Clinical Studies Group"

Name	Department
Dana Andersen, M.D.	Surgery
Susan Anderson, R.N.	Medical Oncology
Yung-chi Cheng, Ph.D.	Pharmacology
José Costa, M.D.	Pathology
Albert Deisseroth, M.D.	Medical Oncology
Vincent T. DeVita, M.D.	Yale Cancer Center
Thomas Duffy, M.D.	Laboratory Medicine
Richard Edelson, M.D.	Dermatology
Barry Kacinsky, M.D.	Therapeutic Radiology
Jonathan Knisely, M.D.	Therapeutic Radiology
Joseph Piepmeier, M.D.	Surgery (Neurosurgery)
Joel Rappeport, M.D.	Internal Medicine
Brian Smith, M.D.	Laboratory Medicine
David Stern, Ph.D.	Pathology
Jack van Hoff, M.D.	Pediatrics
Daniel Zelterman, Ph.D.	Epidemiology and Public Health

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Breast		S94-11472				OCT #27	3	3			
Breast		S94-11688				OCT #28	2	2			
Fat	breast	S94-11688	Frozen in							1	
Breast		S94-12104				OCT #29	2				
Skin	breast	S94-12104	Fresh	1		OCT #29	1				
Breast		S94-12126				OCT #29	1	1			
Breast		S94-12136				OCT #29	1	2			
Breast		S94-12603	Oncofix		1	OCT #30	1	1			
Skin	breast	S94-12603				OCT #30	1				
Breast		S94-12778				OCT #30		4			
Breast		S94-12912				OCT #30	2	3			
Breast		S94-13117	Oncofix	1		OCT #31	1	2			
Breast		S94-13199				OCT #31	1	1			
Breast		S94-13631							Snap #21	1	1
Fat	breast	S94-13779	bottles	2							
Fat	breast	S94-13831	bottles	2							
Skin	breast	S94-13831	Fresh	1							
Breast		S94-13831				OCT #32	1				
Fat	breast	S94-13903	bottle	1							
Fat	breast	S94-14937	bottle	1							
Fat	breast	S94-14937		1							
Fat	breast	S94-14946		1							
Fat	breast	S94-14946		1							
Fat	breast	S94-14978		1							
Fat	breast	S94-14978		1							
Fat	breast	S94-15006		1							
Fat	breast	S94-15025		1							
Fat	breast	S94-15026									
Fat	breast	S94-15046		1							
Fat	breast	S94-15046		2							
Fat	breast	S94-15063									
Breast		S94-15063				OCT #34	1	2			
Skin	breast	S94-15063	Own solution	1							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S94-15115		1							
Fat	breast	S94-15167		1							
Fat	breast	S94-15226		1							
Fat	breast	S94-15249		2							
Skin	breast	S94-15249	Own solution	1							
Breast		S94-15249				OCT #34		1			
Breast		S94-15249				OCT #34		1			
Fat	breast	S94-15394		1							
Skin	breast	S94-15442		1							
Fat	breast	S94-15442		6							
Skin	breast	S94-15442		1							
Fat	breast	S94-15502		1							
Fat	breast	S94-15522		1							
Fat	breast	S94-15539		1							
Fat	breast	S94-15564		1							
Fat	breast	S94-15588		4							
Fat	breast	S94-15643		1							
Fat	breast	S94-15685	Fresh	1							
Fat	breast	S94-15713		1							
Fat	breast	S94-15876		2							
Fat	breast	S94-15938		1							
Breast		S94-15938						1			
Breast		S94-16244				Oct #54	1				
Breast		S94-16244				OCT #37	1	3			
Fat	breast	S94-17274		2							
Breast		S94-17299				OCT #37		2			
Breast		S94-17390				OCT #37		6			
Fat	breast	S94-17390		2							
Fat	breast	S94-17451		1							
Breast		S94-17451							Snap #25	1	1
Fat	breast	S94-17583		1							
Fat	breast	S94-17603		1							
Breast		S94-17659				OCT #38		1			

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S94-17664		3							
Breast		S94-17664	Fresh	1							
Fat	breast	S94-17745		6							
Skin	breast	S94-17745	saline	1							
Fat	breast	S94-17760		2							
Breast		S94-17822				OCT #38	3	3			
Fat	breast	S94-17822		2							
Fat	breast	S94-17899		1							
Breast		S94-17899				OCT #37	2	2			
Breast		S94-17899				OCT #38		1			
Fat	breast	S94-18035		1							
Fat	breast	S94-18109		1							
Fat	breast	S94-18299		1							
Fat	breast	S94-18369		1							
Fat	breast	S94-18406		1							
Fat	breast	S94-18456		1							
Fat	breast	S94-18503		1							
Fat	breast	S94-18654	Frozen in	2							
Breast		S94-18677				OCT #40	2	4		1	1
Fat	breast	S94-18751		1							
Breast		S94-18820				Oct #54	1				
Fat	breast	S94-18820		2							
Breast		S94-18820									
Fat	breast	S94-18915	Formalin	1							
Fat	breast	S94-18915		1							
Fat	breast	S94-18988	Formalin	1							
Fat	breast	S94-18988		1							
Breast		S94-18988				OCT #41	2	7		2	2
Breast		S94-19004				OCT #40	1	2			
Fat	breast	S94-19004		1							
Fat	breast	S94-19163	Formalin	2							
Fat	breast	S94-19163		2							
Breast		S94-19163				OCT #41	2	2			

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S94-19181		1							
Fat	breast	S94-19513		1							
Fat	breast	S94-19538		2							
Skin	breast	S94-19538	Fresh	1							
Fat	breast	S94-19651		5							
Mass	breast	S94-19898				Oct #53	1				
Fat	breast	S94-19898		1							
Fat	breast	S94-19980	Formalin	1							
Fat	breast	S94-20125		1							
Breast		S94-20131				Oct #54	1				
Fat	breast	S94-20131		1							
Fat	breast	S94-20137		1							
Fat	breast	S94-20160		1							
Fat	breast	S94-20225		1							
Fat	breast	S94-20249	Formalin	2							
Fat	breast	S94-20335		1							
Fat	breast	S94-20335	Formalin	1							
Fat	breast	S94-20378		1							
Fat	breast	S94-20378	Formalin	1							
Fat	breast	S94-20650		1			45				
Breast		S94-20650							Snap #28	1	1
Fat	breast	S94-20663		1							
Fat	breast	S94-20947		3							
Fat	breast	S94-20959		1							
Fat	breast	S94-21123		1							
Breast		S94-21123				OCT 50	2	2			
Fat	breast	S94-21124		3							
Fat	breast	S94-21246		2							
Fat	breast	S94-21271		1							
Fat	breast	S94-21331		1							
Fat	breast	S94-21336		4							
Fat	breast	S94-21456		1							
Fat	breast	S94-21472		1							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S94-21791		1							
Breast		S94-21791				OCT #52	2	4		1	1
Fat	breast	S94-21796				OCT #52	1	2			
Fat	breast	S94-21796		1							
Breast		S94-21796				OCT #52	1	2			
Fat	breast	S94-21942		2							
Fat	breast	S94-22017		2							
Fat	breast	S94-22040		2							
Breast		S94-22158							Snap 29	1	1
Fat	breast	S95-238		1							
Fat	breast	S95-274		1							
Fat	breast	S95-373		2							
Fat	breast	S95-437		1							
Fat	breast	S95-469		1							
Fat	breast	S95-478		1							
Fat	breast	S95-631		1							
Fat	breast	S95-652		1							
Fat	breast	S95-714		2							
Fat	breast	S95-749		4							
Breast		S95-792				95-2	1	3			
Fat	breast	S95-792		1							
Fat	breast	S95-840		1							
Fat	breast	S95-876	Formalin								
Breast		S95-876	Oncofix		1	95-2	1	1			
Fat	breast	S95-876		1							
Fat	breast	S95-924	Formalin	1							
Fat	breast	S95-1295		1							
Fat	breast	S95-1352	Fresh	1			3				
Fat	breast	S95-1411	Fresh	2							
Fat	breast	S95-1432	Formalin	1							
Fat	breast	S95-1650		1							
Fat	breast	S95-1650	Formalin	3							
Fat	breast	S95-1650	Formalin	3							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-1753	Fresh	1							
Fat	breast	S95-1753	Fresh	1							
Fat	breast	S95-1769		1							
Fat	breast	S95-1769	Fresh	1							
Fat	breast	S95-1771	Fresh	1							
Fat	breast	S95-1852	Fresh	1							
Fat	breast	S95-1852	Formalin	1							
Fat	breast	S95-1852	Formalin	1							
Breast		S95-1852				95-4	2	3			
Fat	breast	S95-1854	Fresh	1							
Fat	breast	S95-1854	Fresh	1							
Fat	breast	S95-1854									
Fat	breast	S95-1854	Fresh	1							
Fat	breast	S95-1877	Formalin	1							
Fat	breast	S95-1877	Formalin	1							
Fat	breast	S95-1880	Fresh	1							
Fat	breast	S95-1880	Fresh	1							
Fat	breast	S95-1881	Fresh	1							
Fat	breast	S95-1881	Formalin	1							
Fat	breast	S95-1881	Formalin	1							
Fat	breast	S95-1881	Fresh	1							
Fat	breast	S95-2015	Fresh	1							
Fat	breast	S95-2015	Formalin	4							
Fat	breast	S95-2015	Formalin	4							
Fat	breast	S95-2015		1							
Breast		S95-2015	Fresh	1							
Skin	breast	S95-2015	Fresh	2							
Fat	breast	S95-2065	Fresh	1							
Fat	breast	S95-2147	Fresh	1							
Fat	breast	S95-2147	Formalin	1							
Fat	breast	S95-2147	Formalin	1							
Fat	breast	S95-2265	Formalin	1							
Fat	breast	S95-2265	Formalin	1							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Breast		S95-2274									
Fat	breast	S95-2302	Fresh	1							
Breast		S95-2302				95-7	2	2			
Fat	breast	S95-2307	Formalin	1							
Fat	breast	S95-2307	Formalin	1							
Fat	breast	S95-2349	Fresh	4							
Fat	breast	S95-2349	Formalin	2							
Fat	breast	S95-2349	Formalin	2							
Fat	breast	S95-2569	Fresh	1							
Fat	breast	S95-2569	Formalin	2							
Fat	breast	S95-2569	Fresh	1							
Fat	breast	S95-2569	Formalin	2							
Fat	breast	S95-2591	Fresh	1							
Fat	breast	S95-2591	Fresh	1							
Fat	breast	S95-2691	Fresh	2							
Fat	breast	S95-2691	Formalin	4							
Fat	breast	S95-2691	Fresh	2							
Fat	breast	S95-2691	Formalin	4							
Skin	breast	S95-2691	Fresh	2							
Fat	breast	S95-2738	Fresh	1							
Fat	breast	S95-2738	Fresh	1							
Fat	breast	S95-2780	Fresh	1							
Fat	breast	S95-2780	Fresh	1							
Fat	breast	S95-2801	Fresh	1							
Fat	breast	S95-2801	Fresh	1							
Fat	breast	S95-2869	Fresh	1							
Fat	breast	S95-2869	Formalin	2							
Fat	breast	S95-2869	Formalin	3							
Fat	breast	S95-2869	Fresh	1							
Fat	breast	S95-2869	Carnoy	2							
Fat	breast	S95-2897	Fresh	1							
Fat	breast	S95-2897	Formalin	1							
Fat	breast	S95-2897	Formalin	3							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-2897	Fresh	1							
Fat	breast	S95-2897	Carnoy	1							
Skin	breast	S95-2897				95-10	1				
Breast		S95-2897				95-10	1				
Fat	breast	S95-2913	Fresh	1							
Fat	breast	S95-2913	Fresh	1							
Fat	breast	S95-2921	Fresh	1							
Fat	breast	S95-2921	Formalin	4							
Fat	breast	S95-2921	Fresh	1							
Fat	breast	S95-2921	Formalin	4							
Breast	slides	S95-2940						8			
Fat	breast	S95-2940	Formalin	3							
Fat	breast and	S95-2940	Formalin	3							
Breast		S95-2940				95-11	2	2			
Breast	slides	S95-2940				95-13		15			
Fat	breast	S95-2968	Formalin	1							
Fat	breast	S95-2968	Formalin	1							
Breast	slides	S95-2988				95-16		15			
Breast	slides	S95-2988						8			
Fat	breast	S95-2988	Formalin	4							
Fat	breast	S95-2988	Formalin	4							
Breast		S95-2988				95-11	2	2	95-11	1	1
Fat	breast	S95-3070	Fresh	1							
Fat	breast	S95-3070	Fresh	1							
Fat	breast	S95-3139	Fresh	1							
Fat	breast	S95-3139	Fresh	1							
Fat	breast	S95-3257	Fresh	1							
Fat	breast	S95-3257	Fresh	1							
Fat	breast	S95-3289	Formalin	1							
Breast		S95-3320				95-12	1				
Skin	breast	S95-3320				95-12	2				
Breast		S95-3320				95-12	2				
Skin	breast	S95-3320				95-12	2				

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-3320	Fresh	1							
Fat	breast	S95-3320	Formalin	2							
Fat	breast	S95-3320	Fresh	1							
Fat	breast	S95-3341	Formalin	1							
Fat	breast	S95-3341	Formalin	1							
Fat	breast	S95-3341	Formalin	1							
Fat	breast	S95-3456	Fresh	4							
Skin	breast	S95-3456		2							
Fat	breast	S95-3502	Fresh	3							
Breast	slides	S95-3551						8			
Breast		S95-3551	Oncofix	1		95-13		6	95-13		1
Breast		S95-3551				95-13		15			
Fat	breast	S95-3598	Fresh	1							
Fat	breast	S95-3598	Formalin	3							
Skin	breast	S95-3598	RPMI	1							
Fat	breast	S95-3650	Fresh	1							
Fat	breast	S95-3652	Fresh	1							
Fat	breast	S95-3674	Formalin	1							
Fat	breast	S95-3684	Fresh	1							
Breast		S95-3684				95-14	2	2			
Fat	breast	S95-3734	Fresh	1							
Fat	breast	S95-3832	Fresh	1							
Fat	breast	S95-3832	Formalin	1							
Fat	breast	S95-3884	Formalin	1							
Fat	breast	S95-3908	Fresh	2							
Breast		S95-3910							95-16	1	1
Fat	breast	S95-3910	Fresh	1							
Fat	breast	S95-3946	Formalin	1							
Breast	slides	S95-3998				95-17		15			
Breast	slides	S95-3998						8			
Fat	breast	S95-4000	Fresh	1							
Breast	slides	S95-4078				95-17		15			
Breast	slides	S95-4078						8			

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-4078	Fresh	1							
Fat	breast	S95-4078	Fresh	1							
Fat	breast	S95-4102	Fresh	1							
Fat	breast	S95-4102	Fresh	1							
Breast		S95-4153				95-18		7			1
Breast		S95-4153				95-18		7			
Fat	breast	S95-4153	Formalin	3							
Fat	breast	S95-4240	Formalin	2							
Breast		S95-4386				95-19	2	3			
Fat	breast	S95-4400	Fresh	1							
Fat	breast	S95-4466	Fresh	1							
Fat	breast	S95-4467	Fresh	1							
Fat	breast	S95-4492	Formalin	1							
Fat	breast	S95-4510	Formalin	2							
Fat	breast	S95-4537	Formalin	1							
Breast		S95-4556				95-19	3	3			
Fat	breast	S95-4556	Fresh	1							
Fat	breast	S95-4556	Formalin	3							
Fat	breast	S95-4611	Fresh	1							
Fat	breast	S95-4613	Fresh	1							
Fat	breast	S95-4693	Fresh	1							
Skin	breast	S95-4857		1							
Breast		S95-4857		1							
Fat	breast	S95-4857	Fresh	6							
Breast		S95-4893									
Fat	breast	S95-4894	Fresh	3							
Fat	breast	S95-4894	Formalin	2							
Fat	breast	S95-4894	Formalin	6							
Breast		S95-4921	Fresh		1	95-22	1	1			
Breast	slides	S95-4921						23			
Fat	breast	S95-4921	Fresh	1							
Fat	breast	S95-4922	Formalin	3							
Fat	breast	S95-4938	Fresh	1							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Breast	slides	S95-4952						22			
Breast		S95-4954				95-23	2	2			
Breast		S95-4961				95-23	2	2			
Breast		S95-4990				95-23	3	2			
Fat	breast	S95-4990	Fresh	1							
Fat	breast	S95-4990	Formalin	2							
Fat	breast	S95-4996	Fresh	1							
Breast		S95-4997				95-23	2	2			
Breast	slides	S95-4997						23			
Fat	breast	S95-5036	Fresh	1							
Breast		S95-5073				95-24	2	2			
Fat	breast	S95-5073	Fresh	1							
Fat	breast	S95-5073	Formalin	2							
Fat	breast	S95-5128	Formalin	4							
Breast		S95-5152				95-25	2	2			
Fat	breast	S95-5152	Fresh	1							
Fat	breast	S95-5152	Formalin	2							
Fat	breast	S95-5168	Fresh	1							
Skin	breast	S95-5257									
Breast		S95-5257									
Fat	breast	S95-5257	Formalin	2							
Fat	breast	S95-5333	Fresh	1							
Fat	breast	S95-5333	Fresh	1							
Fat	breast	S95-5370	Fresh	1							
Fat	breast	S95-5423	Fresh	1							
Breast	slides	S95-5470				95-30		23			
Fat	breast	S95-5474	Fresh	1							
Fat	breast	S95-5493	Fresh	2							
Fat	breast	S95-5493	Formalin	1							
Fat	breast	S95-5494	Fresh	1							
Fat	breast	S95-5588	Fresh	1							
Fat	breast	S95-5619	Fresh	1							
Breast		S95-5668				95-29	2	2			

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Breast	slides	S95-5668				95-31		23			
Fat	breast	S95-5668	Fresh	1							
Fat	breast	S95-5675	Fresh	1							
Fat	breast	S95-5704	Fresh	1							
Fat	breast	S95-5711	Fresh	1							
Fat	breast	S95-5711	Formalin	1							
Fat	breast	S95-5716	Fresh	1							
Fat	breast	S95-5716	Formalin	1							
Fat	breast	S95-5735	Fresh	1							
Fat	breast	S95-5769	Fresh	1							
Fat	breast	S95-5793	Fresh	2							
Breast		S95-5798				95-32	2	2			
Fat	breast	S95-5894	Formalin	2							
Fat	breast	S95-5905	Formalin	1							
Fat	breast	S95-6017	Formalin	1							
Fat	breast	S95-6029	Fresh	1							
Fat	breast	S95-6038	Formalin	1							
Fat	breast	S95-6157	Fresh	1							
Fat	breast	S95-6213	Fresh	1							
Breast		S95-6255	Oncofix		1	95-33	2	3		1	1
Breast	slides	S95-6255				95-35		23			
Fat	breast	S95-6255	Fresh	1							
Fat	breast	S95-6255	Formalin	1							
Fat	breast	S95-6272	Fresh	1							
Fat	breast	S95-6373	Fresh	1							
Fat	breast	S95-6385	Fresh	1							
Fat	breast	S95-6393	Fresh	1							
Fat	breast	S95-6434	Formalin	4							
Breast	plus N skin	S95-6435	Own solution		1	95-31	2	1			
Fat	breast	S95-6435	Fresh	1							
Fat	breast	S95-6435	Formalin	3							
Breast		S95-6445				95-33	2				
Breast	slides	S95-6445				95-35		23			

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-6445	Fresh	1							
Fat	breast	S95-6445	Formalin	3							
Fat	breast	S95-6550	Fresh	1							
Breast	slides	S95-6657				95-43		8			
Breast		S95-6658				95-34	3	3			
Fat	breast	S95-6658	Fresh	1							
Breast	slides	S95-6658						8			
Breast	slides	S95-6658				95-43		8			
Breast		S95-6664				95-35	2	2			
Fat	breast	S95-6664	Fresh	2							
Fat	breast	S95-6664	Formalin	4							
Fat	breast	S95-6683	Formalin	1							
Fat	breast	S95-6683	Formalin	1							
Breast		S95-6687				95-35	1	1			
Fat	breast	S95-6687	Fresh	1							
Breast	slides	S95-6687						8			
Fat	breast	S95-6733	Fresh	1							
Fat	breast	S95-6822	Fresh	1							
Fat	breast	S95-6850	Fresh	1							
Fat	breast	S95-6869	Fresh	1							
Fat	breast	S95-6871	Fresh	1							
Breast		S95-6930		1			1				
Fat	breast	S95-6930	Fresh	6							
Fat	breast	S95-7178	Fresh	1							
Fat	breast	S95-7178	Formalin	4							
Breast		S95-7192				95-38	1	1			
Skin	breast	S95-7192	Fresh	1							
Breast		S95-7192									
Fat	breast	S95-7192	Fresh	1							
Fat	breast	S95-7192	Formalin	4							
Fat	breast	S95-7206	Fresh	1							
Breast		S95-7215				95-39	1	1			
Fat	breast	S95-7215	Fresh	1							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-7215	Formalin	3							
Fat	breast	S95-7232	Fresh	1							
Breast		S95-7286				95-38	1	1			
Fat	breast	S95-7286	Fresh	1							
Fat	breast	S95-7286	Formalin	3							
Fat	breast	S95-7307	Formalin	1							
Breast		S95-7359									
Fat	breast	S95-7359	Formalin	3							
Fat	breast	S95-7382	Fresh	1							
Fat	breast	S95-7390	Fresh	1							
Fat	breast	S95-7392	Formalin	1							
Fat	breast	S95-7408	Fresh	2							
Breast		S95-7408									
Fat	breast	S95-7451	Fresh	1							
Fat	breast	S95-7467	Fresh	1							
Fat	breast	S95-7528	Fresh	1							
Fat	breast	S95-7534	Fresh	1							
Fat	breast	S95-7542	Fresh	1							
Breast		S95-7583				95-41	2	2			
Fat	breast	S95-7583	Fresh	1							
Breast		S95-7583									
Fat	breast	S95-7583	Formalin	2							
Breast		S95-7599				95-41	2	2			
Fat	breast	S95-7599	Formalin	1							
Fat	breast	S95-7601	Fresh	1							
Breast		S95-7601				95-41	4	2			
Breast		S95-7601									
Fat	breast	S95-7601	Formalin	3							
Fat	breast	S95-7641	Fresh	1							
Breast		S95-7647				95-41	2	2			
Fat	breast	S95-7734	Fresh	1							
Fat	breast	S95-7742	Fresh	1							
Fat	breast	S95-8144	Fresh	1							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-8189	Formalin	2							
Fat	breast	S95-8227	Formalin	3							
Fat	breast	S95-8227	Fresh	1							
Fat	breast	S95-8260	Formalin	6							
Skin	breast	S95-8338	Saline	1							
Breast		S95-8338					6				
Fat	breast	S95-8338	Fresh	4							
Fat	breast	S95-8559	Formalin	3							
Fat	breast	S95-8683	Fresh	1							
Fat	breast	S95-8708	Fresh	1							
Fat	breast	S95-8782	Fresh	1							
Fat	breast	S95-8782	Formalin	2							
Breast		S95-8820				95-47	2	4			
Skin	breast	S95-8820				95-47	1				
Fat	breast	S95-9113	Fresh	1							
Fat	breast	S95-9113	Formalin	2							
Fat	breast	S95-9117	Fresh	1							
Fat	breast	S95-9117	Formalin	3							
Fat	breast	S95-9163	Fresh	1							
Breast	slides	S95-9166				95-50		23			
Breast	slides	S95-9167				95-50		23			
Fat	breast	S95-9193	Fresh	3							
Fat	breast	S95-9193	Formalin	3							
Fat	breast	S95-9258	Fresh	1							
Fat	breast	S95-9272	Fresh	1							
Breast		S95-9272				95-49	1	1			
Breast	slides	S95-9272				95-50		23			
Fat	breast	S95-9300	Fresh	6							
Fat	breast	S95-9300	Formalin	6							
Fat	breast	S95-9316	Fresh	1							
Breast		S95-9486				95-51	1	1			
Fat	breast	S95-9486	Fresh	1							
Fat	breast	S95-9486	Fresh	1							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue type		PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-9488	Formalin	6							
Fat	breast &	S95-9604	Formalin	2							
Fat	breast	S95-9616	Fresh	1							
Fat	breast	S95-9674	Formalin	1							
Fat	breast	S95-9681	Fresh	2							
Fat	breast	S95-9681	Fresh	2							
Skin	breast	S95-9681	RPMI	1							
Breast		S95-9729	Oncofix		1	95-53	6	15			
Fat	breast	S95-9752	Formalin	6							
Fat	breast	S95-9752	Fresh	6							
Fat	breast	S95-9768	Formalin	3							
Fat	breast	S95-9889	Fresh	?							
Fat	breast	S95-9897	Formalin	2							
Fat	breast	S95-9897	Formalin	1							
Fat	breast	S95-9897	Formalin	1							
Fat	breast	S95-9911	Fresh	1							
Fat	breast	S95-9927	Formalin	3							
Fat	breast	S95-9927	Fresh	1							
Breast		S95-9927				95-54		2			
Fat	breast	S95-9980	Formalin	3							
Fat	breast	S95-9980	Fresh	1							
Fat	breast	S95-10061	Formalin	1							
Fat	breast	S95-10126	Formalin	4							
Fat	breast	S95-10126	Fresh	2							
Skin	breast	S95-10126	RPMI	1							
Fat	breast	S95-10241	Formalin	1							
Fat	breast	S95-10276	Formalin	3							
Fat	breast	S95-10276	Fresh	1							
Fat	breast	S95-10286	Formalin	6							
Fat	breast	S95-10286	Formalin	6							
Fat	breast	S95-10286	Fresh	6							
Skin	breast	S95-10286	Fresh	1							
Breast		S95-10286	Fresh	2							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-10404	Formalin	6							
Breast		S95-10463	Formalin		1	95-58	1	1			
Fat	breast	S95-10502	Fresh	1							
Fat	breast	S95-10515	Fresh	1							
Fat	breast	S95-10548	Fresh	1							
Fat	breast	S95-10548	Fresh	1							
Breast		S95-10548				95-59	2	2			
Breast		S95-10548									
Fat	breast	S95-10548	Formalin	3							
Fat	breast	S95-10548	Fresh	3							
Fat	breast	S95-10558	Fresh	2							
Breast		S95-10659				95-60	1	1			
Fat	breast	S95-10659	Fresh	2							
Fat	breast	S95-10659	Formalin	3							
Breast		S95-10659									
Breast		S95-10798				95-60	1	1			
Fat	breast	S95-10798	Formalin	1							
Fat	breast	S95-10798	Fresh	1							
Fat	breast	S95-10800	Fresh	1							
Fat	breast	S95-10807	Formalin	6							
Fat	breast	S95-10893	Fresh	1							
Fat	breast	S95-10909	Fresh	1							
Fat	breast	S95-10925	Fresh	3							
Fat	breast	S95-10950	Fresh	1							
Fat	breast	S95-10956	Formalin	1							
Fat	breast	S95-10956	Fresh	1							
Fat	breast	S95-11124	Formalin	2							
Fat	breast	S95-11124	Formalin	1							
Fat	breast	S95-11124	Fresh	1							
Breast		S95-11124				95-62	1	1			
Fat	breast	S95-11125	Formalin	3							
Breast		S95-11125									
Fat	breast	S95-11166	Formalin	3							

Breast Tissue Collected

July 1, 1994 to June 30,1995

Tissue	type	PCTBO Type & No.	Nonfroz type	Nonfroz normal	Nonfroz tumor	OCT Box	OCT Normal	OCT Tumor	Snap Box	Snap Normal	Snap Tumor
Fat	breast	S95-11240	Fresh	1							
Fat	breast	S95-11240	Fresh	1							
Breast		S95-11240				95-63	1	1			
Fat	breast	S95-11274	Formalin	2							
Fat	breast	S95-11274	Fresh	6							
Fat	breast	S95-11275	Fresh	1							
Fat	breast	S95-11275	Fresh	1							
Fat	breast	S95-11297	Formalin	4							
Fat	breast	S95-11297	Fresh	6							
Fat	breast	S95-11354	Fresh	1							
Breast		S95-11358				95-63		2			
Breast		S95-11445				95-64	2	1			
Fat	breast	S95-11447	Fresh	1							
Fat	breast	S95-11476	Formalin	1							
Breast		S95-11610				95-65	2	2			
Fat	breast	S95-11632	Fresh	1							
Fat	breast	S95-11641	Fresh	1							
Breast		S95-11709							95-66	1	1
Breast		S95-11746				95-65	2	2			
Fat	breast	S95-11765	Fresh	1							
Fat	breast	S95-11770	Fresh	1							
Fat	breast	S95-11776	Fresh	1							
Fat	breast	S95-18820	Formalin	1							
Fat	breast	S95-19538	Formalin								
Fat	breast	S95-19561	Formalin	1							
Fat	breast	S95-20125	Formalin	2							
Fat	breast	S95-20562	Formalin								
TOTALS:		**588 cases**		**747**	**12**		**189**	**559**		**7**	**14**

Appendix 6: Distribution of breast tissues

Tissue Distribution Report

Report Period: 7/1/94 - 6/30/95

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
CARTER									
Breast		S94-12603	7/22/94		1				
Breast		S94-13117	8/1/94	1					
Breast		S95-876	1/17/95		1				
Breast		S95-3551	2/27/95	1					
Breast		S95-6255	4/10/95		1				
Breast		S95-9729	5/31/95		1				
Breast		**9 Case(s) 10 Sample(s)**		**3**	**5**		**1**	**1**	**1**
Total for P.I.		**9 Case(s) 10 Sample(s)**		**3**	**5**		**1**	**1**	**1**
DUBROW									
Breast		S95-6658	4/17/95			1	1		
Breast		**3 Case(s) 4 Sample(s)**				**2**	**2**		
Fat	breast	S94-13779	8/10/94	2					
Fat	breast	S94-13831	8/11/94	2					
Fat	breast	S94-13903	8/12/94	1					
Fat	breast	S94-14937	8/29/94	1					
Fat	breast	S94-14937	8/29/94	1					
Fat	breast	S94-14946	8/29/94	1					
Fat	breast	S94-14946	8/29/94	1					
Fat	breast	S94-14978	8/29/94	1					
Fat	breast	S94-14978	8/29/94	1					
Fat	breast	S94-15006	8/30/94	1					
Fat	breast	S94-15025	8/30/94	1					
Fat	breast	S94-15026	8/30/94						
Fat	breast	S94-15046	8/31/94	1					
Fat	breast	S94-15046	8/31/94	2					
Fat	breast	S94-15063	8/31/94						
Fat	breast	S94-15115	9/1/94	1					
Fat	breast	S94-15167	9/1/94	1					
Fat	breast	S94-15226	9/2/94	1					
Fat	breast	S94-15249	9/24/94	2					
Fat	breast	S94-15394	9/7/94	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S94-15442	9/8/94	6					
Fat	breast	S94-15502	9/9/94	1					
Fat	breast	S94-15522	8/29/94	1					
Fat	breast	S94-15539	9/12/94	1					
Fat	breast	S94-15564	9/9/94	1					
Fat	breast	S94-15588	9/9/94	4					
Fat	breast	S94-15643	9/12/94	1					
Fat	breast	S94-15685	9/13/94	1					
Fat	breast	S94-15713	9/14/94	1					
Fat	breast	S94-15938	9/16/94	1					
Fat	breast	S94-17390	10/10/94	2					
Fat	breast	S94-17451	10/12/94	1					
Fat	breast	S94-17583	10/13/94	1					
Fat	breast	S94-17603	10/12/94	1					
Fat	breast	S94-17664	10/13/94	3					
Fat	breast	S94-17745	10/14/94	6					
Fat	breast	S94-17760	10/7/94	2					
Fat	breast	S94-17822	10/17/94	2					
Fat	breast	S94-17899	10/17/94	1					
Fat	breast	S94-18035	10/19/94	1					
Fat	breast	S94-18109	10/20/94	1					
Fat	breast	S94-18299	10/24/94	1					
Fat	breast	S94-18369	10/25/94	1					
Fat	breast	S94-18406	10/25/94	1					
Fat	breast	S94-18456	10/26/94	1					
Fat	breast	S94-18503	10/27/94	1					
Fat	breast	S94-18654	10/29/94	2					
Fat	breast	S94-18751	10/31/94	1					
Fat	breast	S94-18820	11/1/94	2					
Fat	breast	S94-18915	1/12/95	1					
Fat	breast	S94-18915	11/2/94	1					
Fat	breast	S94-18988	1/12/95	1					
Fat	breast	S94-18988	11/3/94	1					
Fat	breast	S94-19004	11/3/94	1					
Fat	breast	S94-19163	1/12/95	2					
Fat	breast	S94-19163	11/7/94	2					
Fat	breast	S94-19181	11/7/94	1					
Fat	breast	S94-19513	11/11/94	1					
Fat	breast	S94-19538	11/11/94	2					
Fat	breast	S94-19651	11/15/94	5					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S94-19898	11/17/94	1					
Fat	breast	S94-19980	1/12/95	1					
Fat	breast	S94-20125	11/22/94	1					
Fat	breast	S94-20131	11/22/94	1					
Fat	breast	S94-20137	11/21/94	1					
Fat	breast	S94-20160	11/21/94	1					
Fat	breast	S94-20225	11/22/94	1					
Fat	breast	S94-20249	1/12/95	2					
Fat	breast	S94-20335	11/23/94	1					
Fat	breast	S94-20335	1/12/95	1					
Fat	breast	S94-20378	11/25/94	1					
Fat	breast	S94-20378	1/12/95	1					
Fat	breast	S94-20650	11/30/94	1					
Fat	breast	S94-20663	11/29/94	1					
Fat	breast	S94-20947	12/5/94	3					
Fat	breast	S94-20959	12/5/94	1					
Fat	breast	S94-21123	12/4/94	1					
Fat	breast	S94-21124	12/8/94	3					
Fat	breast	S94-21246	12/8/94	2					
Fat	breast	S94-21271	12/8/94	1					
Fat	breast	S94-21331	12/9/94	1					
Fat	breast	S94-21336	12/9/94	4					
Fat	breast	S94-21456	12/12/94	1					
Fat	breast	S94-21472	12/12/94	1					
Fat	breast	S94-21791	12/16/94	1					
Fat	breast	S94-21796	12/16/94	1					
Fat	breast	S94-21942	12/20/94	2					
Fat	breast	S94-22017	12/20/94	2					
Fat	breast	S94-22040	12/21/94	2					
Fat	breast	S95-238	1/5/95	1					
Fat	breast	S95-274	1/6/95	1					
Fat	breast	S95-373	1/5/95	2					
Fat	breast	S95-437	1/10/95	1					
Fat	breast	S95-469	1/10/95	1					
Fat	breast	S95-478	1/10/95	1					
Fat	breast	S95-631	1/12/95	1					
Fat	breast	S95-652	1/12/95	1					
Fat	breast	S95-714	1/13/95	2					
Fat	breast	S95-749	1/13/95	4					
Fat	breast	S95-792	1/16/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-840	1/17/95	1					
Fat	breast	S95-876	1/20/95						
Fat	breast	S95-876	1/17/95	1					
Fat	breast	S95-924	1/20/95	1					
Fat	breast	S95-1295	1/23/95	1					
Fat	breast	S95-1352	1/24/95	1					
Fat	breast	S95-1411	1/24/95	2					
Fat	breast	S95-1650	1/27/95	1					
Fat	breast	S95-1753	1/30/95	1					
Fat	breast	S95-1769	1/30/95	1					
Fat	breast	S95-1854	1/31/95	1					
Fat	breast	S95-1880	1/31/95	1					
Fat	breast	S95-1881	1/31/95	1					
Fat	breast	S95-2015	2/2/95	1					
Fat	breast	S95-18820	1/12/95	1					
Fat	breast	S95-19538	1/12/95						
Fat	breast	S95-19561	1/12/95	1					
Fat	breast	S95-20125	1/12/95	2					
Fat	breast	S95-20562	1/12/95						
Fat		**120 Case(s)**	**164 Sample(s)**	**165**					
Fat - breast		S94-11688	7/8/94					1	
Fat - breast		**1 Case(s)**	**1 Sample(s)**					**1**	
Total for P.I.		**124 Case(s)**	**169 Sample(s)**	**165**		**2**	**2**	**1**	
GUTIERREZ									
Breast		S95-4153	3/7/95						1
Breast		**1 Case(s)**	**1 Sample(s)**						**1**
Total for P.I.		**1 Case(s)**	**1 Sample(s)**						**1**
HANDSCHUMACHER									
Breast		S94-18677	10/31/94					1	1
Breast		S94-21791	12/16/94					1	1
Breast		**6 Case(s)**	**4 Sample(s)**	**1**	**1**		**2**	**2**	**2**
Total for P.I.		**6 Case(s)**	**4 Sample(s)**	**1**	**1**		**2**	**2**	**2**
HAYSLETT									
Skin	breast	S94-15063	8/31/94	1					
Skin		**1 Case(s)**	**1 Sample(s)**	**1**					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Total for P.I.		**1 Case(s)**	**1 Sample(s)**	**1**					
HOCHBERG, R.									
Breast	slides	S95-2940	2/16/95				8		
Breast	slides	S95-2988	3/8/95				8		
Breast	slides	S95-3551	3/8/95				8		
Breast	slides	S95-3998	3/8/95				8		
Breast	slides	S95-4078	3/8/95				8		
Breast	slides	S95-4921	3/21/95				10		
Breast	slides	S95-4952	3/21/95				8		
Breast	slides	S95-4997	3/22/95				8		
Breast	slides	S95-5470	3/31/95				8		
Breast	slides	S95-5668	3/31/95				8		
Breast	slides	S95-6255	4/13/95				8		
Breast	slides	S95-6445	4/13/95				8		
Breast	slides	S95-6658	5/1/95				8		
Breast	slides	S95-6687	5/1/95				8		
Breast	slides	S95-9166	5/25/95				8		
Breast	slides	S95-9167	5/25/95				8		
Breast	slides	S95-9272	5/25/95				8		
Breast		**17 Case(s)**	**138 Sample(s)**				**138**		
Total for P.I.		**17 Case(s)**	**138 Sample(s)**				**138**		
KACINSKI									
Breast		S94-17664	10/13/94	1					
Breast		S95-2015	2/2/95	1					
Breast		S95-4921	3/20/95		1				
Breast	plus N skin	S95-6435	4/12/95		1				
Breast		S95-6930	4/19/95	1					
Breast		S95-10286	6/8/95	2					
Breast		**7 Case(s)**	**8 Sample(s)**	**5**	**2**	**1**			
Total for P.I.		**7 Case(s)**	**8 Sample(s)**	**5**	**2**	**1**			
LONGLEY									
Breast		S95-4857	3/17/95	1					
Breast		**2 Case(s)**	**2 Sample(s)**	**2**					
Skin	breast	S94-12104	7/15/94	1					
Skin	breast	S94-13831	8/11/94	1					
Skin	breast	S94-15442	9/8/94	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Skin	breast	S94-15442	9/8/94	1					
Skin	breast	S95-2015	2/2/95	2					
Skin	breast	S95-3598	2/28/95	1					
Skin	breast	S95-4857	3/17/95	1					
Skin	breast	S95-7192	4/24/95	1					
Skin	breast	S95-9681	5/31/95	1					
Skin	breast	S95-10126	6/6/95	1					
Skin		**14 Case(s)**	**13 Sample(s)**	**15**					
Total for P.I.		**16 Case(s)**	**15 Sample(s)**	**17**					
MILSTONE									
Breast		S95-7601	5/1/95			2			
Breast		S95-8338	5/10/95			6			
Breast		**3 Case(s)**	**9 Sample(s)**		**1**	**8**			
Total for P.I.		**3 Case(s)**	**9 Sample(s)**		**1**	**8**			
MURRAY									
Skin	breast	S94-17745	10/14/94	1					
Skin	breast	S94-19538	11/11/94	1					
Skin		**2 Case(s)**	**2 Sample(s)**	**2**					
Total for P.I.		**2 Case(s)**	**2 Sample(s)**	**2**					
MURREN									
Breast		S94-11472	7/6/94				1		
Breast		S94-12136	7/15/94				1		
Breast		S94-12778	7/26/94				1		
Breast		S94-12912	7/27/94				1		
Breast		S94-15063	8/31/94				1		
Breast		S94-16244	9/21/94				1		
Breast		S94-17822	10/17/94				1		
Breast		S94-17899	10/17/94				1		
Breast		S94-18988	11/3/94				1		
Breast		S94-19004	11/3/94				1		
Breast		S94-21123	12/7/94				1		
Breast		S94-21796	12/16/94				1		
Breast		S95-792	1/16/95				1		
Breast		S95-1852	1/31/95				1		
Breast		**19 Case(s)**	**14 Sample(s)**	**1**	**2**		**14**	**2**	**2**
Fat	breast	S94-21796	12/15/94				1		

Tissue Distribution Report

Report Period: 1994

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat		**1 Case(s)**	**1 Sample(s)**				**1**		
Total for P.I.		**20 Case(s)**	**15 Sample(s)**	**1**	**2**		**15**	**2**	**2**
PARKASH									
Breast		S95-10463	6/12/95		1				
Breast		**1 Case(s)**	**1 Sample(s)**		**1**				
Total for P.I.		**1 Case(s)**	**1 Sample(s)**		**1**				
POBER									
Skin	breast	S95-2691	3/13/95	2					
Skin	breast	S95-3456	2/24/95	2					
Skin	breast	S95-8338	5/10/95	1					
Skin	breast	S95-10286	6/8/95	1					
Skin		**4 Case(s)**	**6 Sample(s)**	**6**					
Total for P.I.		**4 Case(s)**	**6 Sample(s)**	**6**					
SNYDER									
Breast		S93-2570	9/27/94					1	1
Breast		**1 Case(s)**	**2 Sample(s)**					**1**	**1**
Total for P.I.		**1 Case(s)**	**2 Sample(s)**					**1**	**1**
ZHENG									
Fat	breast	S95-1432	1/24/95	1					
Fat	breast	S95-1650	2/14/95	3					
Fat	breast	S95-1650	2/14/95	3					
Fat	breast	S95-1753	1/30/95	1					
Fat	breast	S95-1769	1/30/95	1					
Fat	breast	S95-1771	1/30/95	1					
Fat	breast	S95-1852	1/31/95	1					
Fat	breast	S95-1852	2/13/95	1					
Fat	breast	S95-1852	2/13/95	1					
Fat	breast	S95-1854	1/31/95	1					
Fat	breast	S95-1854	1/31/95	1					
Fat	breast	S95-1877	2/13/95	1					
Fat	breast	S95-1877	2/13/95	1					
Fat	breast	S95-1880	1/31/95	1					
Fat	breast	S95-1881	1/31/95	1					
Fat	breast	S95-1881	2/14/95	1					
Fat	breast	S95-1881	2/14/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-2015	2/2/95	1					
Fat	breast	S95-2015	2/13/95	4					
Fat	breast	S95-2015	2/13/95	4					
Fat	breast	S95-2065	2/2/95	1					
Fat	breast	S95-2147	2/3/95	1					
Fat	breast	S95-2147	2/13/95	1					
Fat	breast	S95-2147	2/13/95	1					
Fat	breast	S95-2265	2/13/95	1					
Fat	breast	S95-2265	2/13/95	1					
Fat	breast	S95-2302	2/7/95	1					
Fat	breast	S95-2307	2/13/95	1					
Fat	breast	S95-2307	2/13/95	1					
Fat	breast	S95-2349	2/7/95	4					
Fat	breast	S95-2349	2/13/95	2					
Fat	breast	S95-2349	2/13/95	2					
Fat	breast	S95-2569	2/10/95	1					
Fat	breast	S95-2569	2/14/95	2					
Fat	breast	S95-2569	2/10/95	1					
Fat	breast	S95-2569	2/14/95	2					
Fat	breast	S95-2591	2/10/95	1					
Fat	breast	S95-2591	2/10/95	1					
Fat	breast	S95-2691	2/13/95	2					
Fat	breast	S95-2691	2/15/95	4					
Fat	breast	S95-2691	2/13/95	2					
Fat	breast	S95-2691	2/15/95	4					
Fat	breast	S95-2738	2/13/95	1					
Fat	breast	S95-2738	2/13/95	1					
Fat	breast	S95-2780	2/14/95	1					
Fat	breast	S95-2780	2/14/95	1					
Fat	breast	S95-2801	2/14/95	1					
Fat	breast	S95-2801	2/14/95	1					
Fat	breast	S95-2869	2/15/95	1					
Fat	breast	S95-2869	2/21/95	2					
Fat	breast	S95-2869	2/28/95	3					
Fat	breast	S95-2869	2/15/95	1					
Fat	breast	S95-2869	2/21/95	2					
Fat	breast	S95-2897	2/15/95	1					
Fat	breast	S95-2897	2/21/95	1					
Fat	breast	S95-2897	2/28/95	3					
Fat	breast	S95-2897	2/15/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-2897	2/21/95	1					
Fat	breast	S95-2913	2/15/95	1					
Fat	breast	S95-2913	2/15/95	1					
Fat	breast	S95-2921	2/17/95	1					
Fat	breast	S95-2921	2/21/95	4					
Fat	breast	S95-2921	2/17/95	1					
Fat	breast	S95-2921	2/21/95	4					
Fat	breast	S95-2940	2/21/95	3					
Fat	breast and	S95-2940	2/21/95	3					
Fat	breast	S95-2968	2/21/95	1					
Fat	breast	S95-2968	2/21/95	1					
Fat	breast	S95-2988	2/21/95	4					
Fat	breast	S95-2988	2/21/95	4					
Fat	breast	S95-3070	2/17/95	1					
Fat	breast	S95-3070	2/17/95	1					
Fat	breast	S95-3139	2/15/95	1					
Fat	breast	S95-3139	2/15/95	1					
Fat	breast	S95-3257	2/21/95	1					
Fat	breast	S95-3257	2/21/95	1					
Fat	breast	S95-3289	2/24/95	1					
Fat	breast	S95-3320	2/22/95	1					
Fat	breast	S95-3320	2/24/95	2					
Fat	breast	S95-3320	2/22/95	1					
Fat	breast	S95-3341	2/22/95	1					
Fat	breast	S95-3341	2/24/95	1					
Fat	breast	S95-3341	2/22/95	1					
Fat	breast	S95-3456	2/24/95	4					
Fat	breast	S95-3502	2/24/95	3					
Fat	breast	S95-3598	2/28/95	1					
Fat	breast	S95-3598	3/3/95	3					
Fat	breast	S95-3650	2/28/95	1					
Fat	breast	S95-3652	2/28/95	1					
Fat	breast	S95-3674	3/3/95	1					
Fat	breast	S95-3684	2/28/95	1					
Fat	breast	S95-3734	3/1/95	1					
Fat	breast	S95-3832	3/2/95	1					
Fat	breast	S95-3832	3/8/95	1					
Fat	breast	S95-3884	3/7/95	1					
Fat	breast	S95-3908	3/3/95	2					
Fat	breast	S95-3910	3/3/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-3946	3/7/95	1					
Fat	breast	S95-4000	3/6/95	1					
Fat	breast	S95-4078	3/7/95	1					
Fat	breast	S95-4078	3/7/95	1					
Fat	breast	S95-4102	3/7/95	1					
Fat	breast	S95-4102	3/7/95	1					
Fat	breast	S95-4153	3/15/95	3					
Fat	breast	S95-4240	3/20/95	2					
Fat	breast	S95-4400	3/10/95	1					
Fat	breast	S95-4466	3/13/95	1					
Fat	breast	S95-4467	3/13/95	1					
Fat	breast	S95-4492	3/16/95	1					
Fat	breast	S95-4510	3/16/95	2					
Fat	breast	S95-4537	3/15/95	1					
Fat	breast	S95-4556	3/14/95	1					
Fat	breast	S95-4556	3/20/95	3					
Fat	breast	S95-4611	3/14/95	1					
Fat	breast	S95-4613	3/14/95	1					
Fat	breast	S95-4693	3/15/95	1					
Fat	breast	S95-4857	3/17/95	6					
Fat	breast	S95-4894	3/17/95	3					
Fat	breast	S95-4894	3/21/95	2					
Fat	breast	S95-4894	3/24/95	6					
Fat	breast	S95-4921	3/20/95	1					
Fat	breast	S95-4922	3/24/95	3					
Fat	breast	S95-4938	3/20/95	1					
Fat	breast	S95-4990	3/21/95	1					
Fat	breast	S95-4990	3/24/95	2					
Fat	breast	S95-4996	3/21/95	1					
Fat	breast	S95-5036	3/21/95	1					
Fat	breast	S95-5073	3/22/95	1					
Fat	breast	S95-5073	3/27/95	2					
Fat	breast	S95-5128	3/27/95	4					
Fat	breast	S95-5152	3/22/95	1					
Fat	breast	S95-5152	3/29/95	2					
Fat	breast	S95-5168	4/7/95	1					
Fat	breast	S95-5257	3/29/95	2					
Fat	breast	S95-5333	3/27/95	1					
Fat	breast	S95-5333	3/27/95	1					
Fat	breast	S95-5370	3/27/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-5423	3/28/95	1					
Fat	breast	S95-5474	3/28/95	1					
Fat	breast	S95-5493	3/28/95	2					
Fat	breast	S95-5493	4/3/95	1					
Fat	breast	S95-5494	3/28/95	1					
Fat	breast	S95-5588	3/30/95	1					
Fat	breast	S95-5619	3/30/95	1					
Fat	breast	S95-5668	3/31/95	1					
Fat	breast	S95-5675	3/31/95	1					
Fat	breast	S95-5704	3/31/95	1					
Fat	breast	S95-5711	3/31/95	1					
Fat	breast	S95-5711	4/5/95	1					
Fat	breast	S95-5716	3/31/95	1					
Fat	breast	S95-5716	4/5/95	1					
Fat	breast	S95-5735	3/31/95	1					
Fat	breast	S95-5769	4/7/95	1					
Fat	breast	S95-5793	4/7/95	2					
Fat	breast	S95-5894	4/19/95	2					
Fat	breast	S95-5905	4/11/95	1					
Fat	breast	S95-6017	4/12/95	1					
Fat	breast	S95-6029	4/5/95	1					
Fat	breast	S95-6038	4/11/95	1					
Fat	breast	S95-6157	4/7/95	1					
Fat	breast	S95-6213	4/7/95	1					
Fat	breast	S95-6255	4/10/95	1					
Fat	breast	S95-6255	4/11/95	1					
Fat	breast	S95-6272	4/10/95	1					
Fat	breast	S95-6373	4/11/95	1					
Fat	breast	S95-6385	4/11/95	1					
Fat	breast	S95-6393	4/11/95	1					
Fat	breast	S95-6434	4/18/95	4					
Fat	breast	S95-6435	4/12/95	1					
Fat	breast	S95-6435	4/18/95	3					
Fat	breast	S95-6445	4/12/95	1					
Fat	breast	S95-6445	4/18/95	3					
Fat	breast	S95-6550	4/13/95	1					
Fat	breast	S95-6658	4/17/95	1					
Fat	breast	S95-6664	4/17/95	2					
Fat	breast	S95-6664	4/25/95	4					
Fat	breast	S95-6683	4/18/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-6683	4/25/95	1					
Fat	breast	S95-6687	4/17/95	1					
Fat	breast	S95-6733	4/18/95	1					
Fat	breast	S95-6822	4/18/95	1					
Fat	breast	S95-6850	4/19/95	1					
Fat	breast	S95-6869	4/19/95	1					
Fat	breast	S95-6871	4/19/95	1					
Fat	breast	S95-6930	4/19/95	6					
Fat	breast	S95-7178	4/24/95	1					
Fat	breast	S95-7178	4/28/95	4					
Fat	breast	S95-7192	4/24/95	1					
Fat	breast	S95-7192	4/28/95	4					
Fat	breast	S95-7206	4/24/95	1					
Fat	breast	S95-7215	4/25/95	1					
Fat	breast	S95-7215	4/28/95	3					
Fat	breast	S95-7232	4/25/95	1					
Fat	breast	S95-7286	4/25/95	1					
Fat	breast	S95-7286	5/2/95	3					
Fat	breast	S95-7307	4/25/95	1					
Fat	breast	S95-7359	5/3/95	3					
Fat	breast	S95-7382	4/26/95	1					
Fat	breast	S95-7390	4/26/95	1					
Fat	breast	S95-7392	4/26/95	1					
Fat	breast	S95-7408	4/27/95	2					
Fat	breast	S95-7451	4/27/95	1					
Fat	breast	S95-7467	4/27/95	1					
Fat	breast	S95-7528	4/28/95	1					
Fat	breast	S95-7534	4/28/95	1					
Fat	breast	S95-7542	4/28/95	1					
Fat	breast	S95-7583	5/1/95	1					
Fat	breast	S95-7583	5/8/95	2					
Fat	breast	S95-7599	5/5/95	1					
Fat	breast	S95-7601	5/1/95	1					
Fat	breast	S95-7601	5/5/95	3					
Fat	breast	S95-7641	5/1/95	1					
Fat	breast	S95-7734	5/2/95	1					
Fat	breast	S95-7742	5/2/95	1					
Fat	breast	S95-8144	5/8/95	1					
Fat	breast	S95-8189	5/23/95	2					
Fat	breast	S95-8227	5/25/95	3					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-8227	5/9/95	1					
Fat	breast	S95-8260	5/23/95	6					
Fat	breast	S95-8338	5/10/95	4					
Fat	breast	S95-8559	5/25/95	3					
Fat	breast	S95-8683	5/16/95	1					
Fat	breast	S95-8708	5/16/95	1					
Fat	breast	S95-8782	5/16/95	1					
Fat	breast	S95-8782	5/23/95	2					
Fat	breast	S95-9113	5/22/95	1					
Fat	breast	S95-9113	5/30/95	2					
Fat	breast	S95-9117	5/22/95	1					
Fat	breast	S95-9117	5/30/95	3					
Fat	breast	S95-9163	5/23/95	1					
Fat	breast	S95-9193	5/23/95	3					
Fat	breast	S95-9193	5/30/95	3					
Fat	breast	S95-9258	5/25/95	1					
Fat	breast	S95-9272	5/24/95	1					
Fat	breast	S95-9300	5/24/95	6					
Fat	breast	S95-9300	6/1/95	6					
Fat	breast	S95-9316	5/24/95	1					
Fat	breast	S95-9486	5/26/95	1					
Fat	breast	S95-9486	5/26/95	1					
Fat	breast	S95-9488	6/2/95	6					
Fat	breast & axillary	S95-9604	6/15/95	2					
Fat	breast	S95-9616	5/30/95	1					
Fat	breast	S95-9674	6/8/95	1					
Fat	breast	S95-9681	5/31/95	2					
Fat	breast	S95-9681	5/31/95	2					
Fat	breast	S95-9752	6/7/95	6					
Fat	breast	S95-9752	5/31/95	6					
Fat	breast	S95-9768	6/7/95	3					
Fat	breast	S95-9889	6/2/95	?					
Fat	breast	S95-9897	6/13/95	2					
Fat	breast	S95-9897	6/12/95	1					
Fat	breast	S95-9897	6/9/95	1					
Fat	breast	S95-9911	6/2/95	1					
Fat	breast	S95-9927	6/13/95	3					
Fat	breast	S95-9927	6/2/95	1					
Fat	breast	S95-9980	6/13/95	3					
Fat	breast	S95-9980	6/5/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-10061	6/9/95	1					
Fat	breast	S95-10126	6/13/95	4					
Fat	breast	S95-10126	6/6/95	2					
Fat	breast	S95-10241	6/9/95	1					
Fat	breast	S95-10276	6/14/95	3					
Fat	breast	S95-10276	6/8/95	1					
Fat	breast	S95-10286	6/13/95	6					
Fat	breast	S95-10286	6/13/95	6					
Fat	breast	S95-10286	6/8/95	6					
Fat	breast	S95-10404	6/12/95	6					
Fat	breast	S95-10502	6/12/95	1					
Fat	breast	S95-10515	6/13/95	1					
Fat	breast	S95-10548	6/13/95	1					
Fat	breast	S95-10548	6/13/95	1					
Fat	breast	S95-10548	6/23/95	3					
Fat	breast	S95-10548	6/20/95	3					
Fat	breast	S95-10558	6/13/95	2					
Fat	breast	S95-10659	6/14/95	2					
Fat	breast	S95-10659	6/21/95	3					
Fat	breast	S95-10798	6/23/95	1					
Fat	breast	S95-10798	6/16/95	1					
Fat	breast	S95-10800	6/16/95	1					
Fat	breast	S95-10807	6/20/95	6					
Fat	breast	S95-10893	6/19/95	1					
Fat	breast	S95-10909	6/20/95	1					
Fat	breast	S95-10925	6/19/95	3					
Fat	breast	S95-10950	6/20/95	1					
Fat	breast	S95-10956	6/23/95	1					
Fat	breast	S95-10956	6/20/95	1					
Fat	breast	S95-11124	6/29/95	2					
Fat	breast	S95-11124	6/21/95	1					
Fat	breast	S95-11124	6/21/95	1					
Fat	breast	S95-11125	6/27/95	3					
Fat	breast	S95-11166	6/28/95	3					
Fat	breast	S95-11240	6/23/95	1					
Fat	breast	S95-11240	6/23/95	1					
Fat	breast	S95-11274	6/28/95	2					
Fat	breast	S95-11274	6/23/95	6					
Fat	breast	S95-11275	6/23/95	1					
Fat	breast	S95-11275	6/23/95	1					

Tissue Distribution Report

Report Period:

Investigator	Tissue type	Number	Date	Non-Frozen Normal	Non-Frozen Tumor	OCT Normal Whole	OCT Tumor Whole	Snap Normal Whole	Snap Tumor Whole
Fat	breast	S95-11297	6/28/95	4					
Fat	breast	S95-11297	6/23/95	6					
Fat	breast	S95-11354	6/26/95	1					
Fat	breast	S95-11447	6/27/95	1					
Fat	breast	S95-11476	6/27/95	1					
Fat	breast	S95-11632	6/28/95	1					
Fat	breast	S95-11641	6/28/95	1					
Fat	breast	S95-11765	6/30/95	1					
Fat	breast	S95-11770	6/30/95	1					
Fat	breast	S95-11776	6/30/95	1					
Fat		**307 Case(s)**	**541 Sample(s)**	**541**					
Total for P.I.		**307 Case(s)**	**541 Sample(s)**	**541**					
Grand Totals		**519 Case(s)**	**923 Sample(s)**	**743**	**12**	**11**	**158**	**7**	**7**

Appendix 7: DNA isolation and evaluation

A. Tissue section preparation
    a. 1-3 25µ sections per sample collected into sterile 1.5ml tube
    b. Fresh blade for each sample
    1. Fresh/frozen OCT-embedded tissue:
        a. Add 1.0ml sterile water, invert several times
        b. Spin 13,000xg 10min; aspirate diluted OCT
    2. Paraffin-embedded tissue:
        a. Add 1.0ml xylene, Warm at 37°C for 30min; Spin at 13,000xg for 10min
        b. Repeat twice with 10min incubations
        c. Add 1.0 ml ethanol; Mix; Spin 10min; Aspirate; Repeat twice
B. Tissue digestion by 500µg/ml Proteinase K
C. DNA extraction
    1. Add 500µl Tris-equilibrated phenol to tissue pellet; Agitate 15min
    2. Spin at 13,000xg for 10min; Save aqueous layer
    3. Extract with 500µl Phenol:Chloroform in Phase Lock Gel™ (light) tube;
    4. Spin 7,000xg for 3min; Save aqueous layer
    5 Extract twice with 500µl chloroform
    6. To aqueous extract add 0.1 volume 3.0M NaOAc and 2.0 volumes 100%EtOH
    7. Allow DNA precipitation at -20°C for 2 - 20 hours
    8. Spin 13,000xg for 10min; Decant; Wash with 70% EtOH
    9. Resuspend in TE, pH 8.0 with incubation at 55°C overnight
D. Evaluation of DNA
    1. Measure A_{260}/A_{280} of 1:100 dilution in TE, pH 8.0
    2. Visualize `100ng' (based on #1) in a 0.8% LE Agarose/TAE gel containing 2µg/ml EtBr with commercial genomic standards run in parallel
    3 Adjust concentration of stock to 20-100ng/µl based on both evaluations
    4. PCR 50ng for -globin sequence to measure utility of DNA for amplification

Appendix 8: Ki-ras Exon 1 (codons 12 & 13) Analysis

PCR for SSCP

PCR primers for exon 1 of Ki-ras are as follows: (+) strand R53 5'-GACTGAATATAAACTTGTGG-3' and (-) strand or R34 5'-CTGTATCAAAGAATGGTCCT-3'. Input DNA was purified by organic extraction and quantitated spectrophotometrically; 1.0 - 100ng [300 - 30,000 targets] of genomic DNA or 1.0 - 10.0pg [300,000 - 3,000,000 targets] control plasmid were added to each PCR. Thirty microliter reactions contained 0.2μM each primer, 50μM each dNTP 0.8U AmpliTaq polymerase (Perkin-Elmer, Corp., Wilton, CT)in Buffer II containing 2.5mM $MgCl_2$. After a 3min hot start at 98°C, 5 cycles of amplification (96°C/30sec - 56°C/45sec - 72°C/45sec), followed by 25 - 35 cycles of amplification (93°C/20sec - 59°C/20sec - 72°C/30sec) was done in a HybeAid Omnigene (National LabNet, ,NJ). Agarose gel electrophoresis, using GelMarker™ (Research Genetics, Huntington, AL) allowed estimation of PCR product quantity and size. Each SSCP evaluation used approximately 30ng of PCR product.

SSCP

Samples were denatured by adding 2.5 - 5.0 volumes of denaturing dye solution (950μl formamide, 2μl 5.0N NaOH, 25μl 0.1% brom phenol blue and 25μl 0.1% xylene cyanol) and heating at 95°C for 5 minutes. After quick chill on ice, samples were loaded onto a denaturing gel composed of 1X MDE (FMC Bioproducts, Rockland, ME) in 0.5X TBE and electrophoresed at 18°C constant temperature and 400V constant current; time varied with gel apparatus used. Gels cast in 14 X 16 cm format were run for 4 hours in an SE600 vertical gel apparatus (Hoefer Pharmacia Biotech Inc., San Francisco, CA); gel temperature was regulated by contact of the the gel plates with the circulating buffer. Minigels, 8 X 10cm, were run for 1 hour in CoolGel ThermMaster (BioTherm, Fairfax, VA) and gel temperature was monitored directly. [PUBLICATION IN PREPARATION]

Allele-Specific PCR

PCR is performed with (+)-strand 17-mer primers having the 3'-terminal base complementary to each one of the possible bases of codon 12 or 13 of Ki-ras and a generic (-)-strand primer, 5'-AATGGTCCTGCACCAGTAAT-3'. [Benhattar J, Losi L, Chaubert P, Givel JC, Costa J. (1993) Frequency and prognostic significance of K-ras mutations in colorectal carcinoma. Gastroenterol;104:1044-8.] Ethidium bromide/agarose gel analysis allows determination of the presence of mutated Ki-ras in tumor samples. Internal control for the PCR is provided by globin primers, present at 0.2X concentration.

Appendix 9: Immunostaining Procedure, Frozen Specimen

Day 1

Cut sections at 6 microns, place on silane-coated slides, fix for 10 minutes in cold (-20° C) acetone, and allow to dry overnight at room temperature.

Day 2

1. Transfer slides quickly to a bath of PBS.
   - wash in PBS for an additional 2 changes.
   - tissue should NOT be allowed to dry after this step

2. Block in 2% BSA/PBS for 30 minutes.
   - wipe around sections WITHOUT allowing tissue to dry out.

3. Normal suppresser serum ~ 30 minutes.
   - aspirate serum WITHOUT leaving tissue dry.

4. Primary Ab: pipette 1 to 2 drops of the primary antibody onto sections and incubate 60 min. at RT (for most 1° antibodies) OR overnight at 4°C.
   - incubate slides in a humidity chamber for either time.

Day 2 or 3

5. Rinse slides:
   - 3x - PBS
   - 1x - 0.01% Triton X-100/PBS
   - 1x - PBS

6. Place in bath of 2° Ab:
   - 20 minutes - anti-mouse
   - 15 minutes - anti-rabbit

7. Rinse as in Step 5.

8. Quench in 1.0% H_2O_2/PBS for 10 min.

9. Rinse 3x - PBS

10. Label [3° - Streptavidin-peroxidase (SAP)]:
    - 20 min. monoclonal primary Ab
    - 15 min. polyclonal primary Ab

11. Rinse:
    - 3x - PBS
    - 3x - 0.01% Triton/PBS
    - let slides sit in lst Triton/PBS for 2 minutes prior to Step 12

12. Chromogen - diaminobenzidine tetrahydrochloride (DAB), **carcinogen!!**

    DAB - (must be filtered before use)

- place slides into DAB bath. After 2 minutes, remove control slide, **rinse briefly in running DH_2O**, and check under the microscope for proper color development. Estimate additional time required for experimental slides.

If reaction time was not sufficient:
- Place slides back into 0.01% Triton/PBS bath for 2 min.
- then place back into DAB bath

13. After reaction time is sufficient
    - allow slides to rise in running DH_2O for 5 minutes.

14. Counter stain:
    - Mayer's hematoxylin - 30 seconds
      rinse in tap H_2O 2 to 3 minutes
    - Ammonia H_2O - dip until blue
      rinse in tap H_2O 2 to 3 minutes

15. Place slides into the following baths:
    - 100% EtOH
    - 100% EtOH
    - 100% EtOH
    - 50/50 - 100% EtOH/Xylene
    - Xylene
    - Xylene
    - Xylene

16. Coverslip with a permanent mounting media (i.e. Permount)

Appendix 10: Protocol for amplification of p53 gene in cancer tissues

DNA extraction:
1. **Paraffin-embedded, formalin-fixed tissues**: Tumoral regions (under histological control) were removed from paraffin blocks by scraping with a sterile scalpel and boiled at 100°C for 10 min in TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH8).

2. **Frozen tissues**: DNA can be extracted by a clasical enzymatic digestion and phenol-chloroforme extraction with ethanol precipitation or by using a Elu-Quick DNA Purification Kit (Schleider & Schuell, Keene NH). According to the manufacturer's instructions, the complete process is carried out in one hour.

PCR reaction:
Exons 5 to 9 are amplified separately.
Each PCR is performed in 30 µl final volume, containing:

- 3 µl DNA
- 0.5 µM each primer*
- 250 µM dNTP mixture
- 50 mM KCL
- 10 mM Tris-HCl, pH 8.3
- 1.75 mM $MgCl_2$
- 0.8 units Taq DNA Polymerase

Mixtures are subjected to 35 cycles of amplification in a Perkin Elmer DNA Thermal Cycler model 9600, consisting of :

- 30 sec 94°C
- 45 sec 55°C (exons 7,8 and 9), 58°C (exon 5) or 60°C (exon 6)
- 45 sec 73°C

Primers used in PCR amplification:

Exon 5:	5'-CCGTGTTCCAGTTGCTTTAT-3' 5'-ACCTCTCTGCTGTCCCGA-3'
Exon 6:	5'-GGGCTGGTTGCCCAGGGT-3' 5'-ACTCCAGACCAAACGTTGA-3'
Exon 7:	5'-CCACAGGTCTCCCCAAGG-3' 5'-CAGTCCTCGGTGAACGGT-3'
Exon 8:	5'-CCTATCCTGAGTAGTGGTAA-3' 5'-TGTTCTTCGCCACCTCCT-3'
Exon 9:	5'-ACCTTTCCTTGCCTCTTTC-3' 5'-CGGCATTTTGAGTGTTAGAC-3'

NON-RADIOACTIVE SSCP ANALYSIS OF PCR PRODUCTS

1 to 4 µl (5-20 ng) of product of amplification are denatured in 50 mM NaOH and 1 mM EDTA at 50°C for 10 min in a final volume of 10 µl. After the addition of 1.5 µl of formamide dye, samples are loaded in an 10% nondenaturing polyacrylamide gel (49:1 acrylamide to bisacrylamide) or MDE gel (Hydro-Link, AT Biochem, Malvern, PA).

Electrophoresis is performed on a vertical gel (160X140X0.75 mm) in a Hoefer Scientific SE600 apparatus (San Francisco, CA) at 20 V/cm in 0.5X TBE for five hours. The temperature is maintained at 18-20°C with constant recirculation of the buffer between the upper and the lower chambers.

The gels are stained using a standard silver technique:

- Fixation in Acetic acid 10% for 20 min
- Rinse in dd water for 2 min, 3 times
- Silver impregnation in a silver solution (1.5 g/L $AgNO_3$, 0.056% formaldehyde) for 30 min
- Rinse in deionized water 15 sec
- Image development in a solution of 30 g/L Na_2CO_3, 0.056% formaldehyde, 400µg/L sodium thiosulfate used at about 8°C for 2-10 min
- Stop the reaction with acetic acid 10%

Finally, the gels are dryed in a Byo Rad Gel Dryer, model 583 at 80°C for 2 hours.